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Artigo

APORPHINE ALKALOIDS FROM *Ocotea macrophylla* (LAURACEAE)

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Four aporphine alkaloids from the wood of *Ocotea macrophylla* (Lauraceae) were isolated and characterized as (*S*)-3-methoxy-nordomesticine (**1**), (*S*)-*N*-ethoxycarbonyl-3-methoxy-nordomesticine (**2**), (*S*)-*N*-formyl-3-methoxy-nordomesticine (**3**) and (*S*)-*N*-methoxycarbonyl-3-methoxy-nordomesticine (**4**); alkaloids 2-4 are being report for the first time. The structure the isolated compounds were determined based on their spectral data and by comparison of their spectral data with values described in literature. The alkaloid fraction and compound **1** showed antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* and also compound **1** showed antimicrobial activity towards *Staphylococcus aureus*, *Enterococcus faecalis* as well.

Keywords: *Ocotea macrophylla;* aporphine alkaloids; Lauraceae.

INTRODUCTION

The *Ocotea* genus (Lauraceae) includes more than 350 species found in the American continent and southern Africa.[1,2] In Colombia, there are 35 *Ocotea* species distributed throughout the country mainly in the Andean forests.[3] In traditional medicine, some *Ocotea* species shown different applications. *O. quixos* is used as disinfectant, local anesthetic and anti-diarrheic.[4] *O. lancifolia* is used as antiparasitic, and *O. caparrapi* is used to treat insect bites, snake bites, bronchitis, and cancerous tumors.[5,6]

Chemically, the *Ocotea* genus is known mainly as a source of metabolites type furofuran[7] and tetrahydrofuran lignans,[8] bicyclo[3.2.1] octane[9] and benzofuran neolignans,[10] and benzylisoquinoline[11] and aporphine alkaloids.[5] In previous studies, four aporphine alkaloids from wood of *Ocotea macrophylla* were isolated and identified as nantenine, glaucine, isocorydine and dehydronantenine.[12,13] In this paper, we describe isolation and structural determination of three new aporphine alkaloids **2-4** besides (S)- 3-methoxy-nordomesticine **1** and the reports antibacterial and antifungal activities of compounds.

RESULT AND DISCUSSION

The ethanolic extract from the stem of *O. macrophylla* was subjected to an acid-base extraction to obtain an alkaloidal fraction, that was further subjected to fractionation and purification by chromatographic methods leading to the isolation of four alkaloids: (*S*)-3-methoxy-nordomesticine (**1**), (*S*)-*N*-ethoxycarbonyl-3-methoxy-nordomesticine (**2**), (*S*)-*N*-formyl-3-methoxy-nordomesticine (**3**) and (*S*)-*N*-methoxycarbonyl-3-methoxy-nordomesticine **(4).** The structures of the alkaloids **1-4** are shown in Figure 1 and spectroscopic data in the Table 1.

Alkaloid **1** was obtained as a yellowish oil that gives a positive reaction to Dragendorff reagent, which suggests the presence of an alkaloid. The molecular formula of **1** was established as $C_{19}H_{19}NO_5$ from HRESIMS at *m/z* 340.1178 $[M-H]^-$, indicating eleven degrees of insaturation. Its UV spectrum showed maximum absorptions at 224 and 308 nm, characteristic of aporphine alkaloids,[14] and the IR spectrum displayed hydroxyl (3404 cm^{-1}), aromatic (1585 and 1467 cm^{-1}), and methylenedioxy (1039 and 935 cm^{-1}) absorptions.[15] The ^1H NMR spectrum shows typical signals for aporphine alkaloids at:

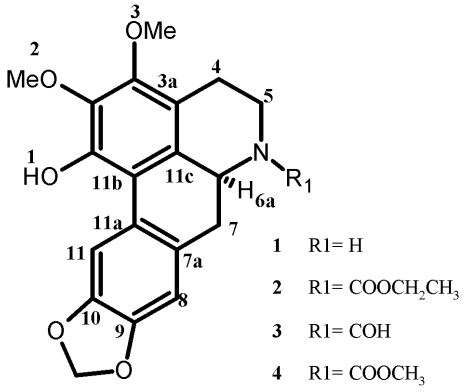

Figure 1. *Basic structure of isolated aporphine alkaloids*

δ 4.74 (1H, *dd, J*=3.9 and 13.8 Hz, H-6a), 3.73 (1H, *ddd, J*=12.1, 4.3 and 2.1 Hz, H-5), 3.24 (1H, *ddd, J*=12.1, 8.4 and 3.6 Hz, H-5), 2.93 (2H, *m,* H-3,H-7) and 2.64 (2H, *m,* H-3,H-7). Also, two singlets were present in the aromatic region at δ 7.90 (H-11), 6.70 (H-8) besides the hydroxyl group at 6.30, as well as signals for a methylenedioxy group at δ 5.95 (1H, *d*, *J*=1.3 Hz) and 5.94 (1H, *d, J*=1.3 Hz)[16] and two methoxyl groups at δ 3.97 and 3.87 (3H, each s). The ^{13}C NMR and DEPT showed spectra of **1** showed 19 signals, corresponding to two methoxyl groups at δ 60.9 and 60.3; four methylene groups at δ 100.9, 43.9, 35.1 and 23.8, where the first signal corresponds to a methylenedioxy group; three methines at δ 108.5, 108.6, and 52.8, and ten quaternary carbons, whose chemical shift are found in Table 1.

The signal at δ_H 6.30 (1H, *s*) showed no connectivity in the HMQC experiment, indicative of the presence of a phenolic OH group, supported by IR. Analysis of the HMBC spectrum allowed the location of substituents, according to the correlations observed between the signals at δ_H 5.94 and 5.95 (for the methylenedioxy group) with the signals at δ_C 145.9 (C-9) and 146.2 (C-10), and these last two signals with the protons at δ_H 6.70 (H-8) and 7.90 (H-11), respectively, suggesting the presence of a methylenedioxy group at positions 9 and 10 and two hydrogens on the aromatic ring in a *para* orientation. The presence of the hydroxyl group at position 1, was determined using correlations between the signals at δ_H 6.30 and δ_C 116.5, assigned to C-11b. The location of the methoxyl groups in positions C-2 and C-3, were assigned according to the correlation of

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Table 1. ^{1}H and ^{13}C NMR spectroscopic assignments of **1-4** in ($CDCl_3$, ^{1}H NMR: 400 MHz and ^{13}C NMR: 100 MHz)

Position	Alkaloid 1		Alkaloid 2		Alkaloid 3a		Alkaloid 3b		Alkaloid 4	
	δ_H (mult., *J*,Hz)	δ_C	δ_H (mult., *J*,Hz)	δ_C	δ_H (mult., *J*,Hz)	δ_C	δ_H (mult., *J*,Hz)	δ_C	δ_H (mult., *J*,Hz)	δ_C
1		144.8		144.8		145.2		145.0		144.8
2		138.4		138.5		139.0		138.7		138.5
3		147.8		147.9		147.8		148.3		147.9
3a		118.5		119.4		118.3		119.3		119.3
4	2.93 (*m*) 2.64 (*m*)	23.8	2.90 (*m*) 2.53 (*m*)	23.5	3.04 (*m*)	24.1	3.04 (*m*)	23.0	2.95 (*m*) 2.52 (*m*)	23.4
5	3.73 (*ddd*, 12.1, 4.3, 2.1) 3.24 (*ddd,* 12.1,8.4,3.6)	43.9	4.45 (*d,* 11.8) 2.95 (*m*)	38.6	3.82 (*ddd,* 12.7, 4.7, 1.7) 3.31 (*dt*, 12.7, 2.7)	41.8	3.05 (*m*) 2.72 (*m*)	37.0	4.43 (*m*) 2.87 (*m*)	38.7
6a	4.74 (*dd,* 13.8, 3.9)	52.8	4.76 (*dd,* 13.5, 4.2)	51.8	4.94 (*dd,* 13.6, 4.2)	49.6	4.46 (*m*)	53.7	4.71 (*d,* 11.2)	51.8
7	2.93 (*m*) 2.64 (*m*)	35.1	2.78 (*m*)	34.8	2.95 (*m*) 2.78 (*m*)	33.7	3.04 2.66 (*m*)	35.9	2.95 (*m*) 2.52 (*m*)	34.0
7a		130.0		130.2		129.6		128.8		130.1
8	6.70 (*s*)	108.5	6.74 (*s*)	108.5	6.76 (*s*)	108.8	6.74(*s*)	108.4	6.74 (*s*)	108.5
9		145.9		146.0		146.1		145.2		145.9
10		146.2		146.3		146.4		147.7		146.2
11	7.90 (*s*)	108.6	7.94 (*s*)	108.8	7.93 (*s*)	108.7	7.94(*s*)	108.9	7.94 (*s*)	108.7
11a		125.2		125.3		125.0				125.3
11b		116.5		116.4		116.6		117.3		116.3
11c		129.9		129.1		128.8				128.9
OH	6.30 (*s*)		6.32 (*s*)		6.33 (*s*)		6.33(*s*)		6.44 (*s*)	
2-OCH_3	3.97 (*s*)	60.9	3.96 (*s*)	61.0	3.96 (*s*)	61.0	3.97(*s*)	61.0	3.95 (*s*)	60.9
3-OCH_3	3.87 (*s*)	60.3	3.86 (*s*)	60.4	3.86 (*s*)	60.5	3.86(*s*)	60.4	3.85 (*s*)	60.4
O-CH_2-O	5.95 (*d,*1.3) 5.94 (*d,*1.3)	100.8	5.97 (*d,*1.4) 5.96 (*d,*1.4)	100.9	5.97 (*d*, 1.3) 5.96 (*d*, 1.3)	100.9	5.99 (*d*, 1.4) 5.97 (*d*, 1.4)	101.0	5.96 (*d,* 1.3) 5.95 (*d,*1.3)	100.8
C=O				158.8	8.25 (*s)*	161.9	8.37 (*s)*	161.8		155.8
-OCH_3			4.23 (*m*)	61.0						
-OCH_2									3.76 (*s*)	52.6
CH_3			1.29 (*t,* 7.10)	14.8						

J values (Hz) are in parenthesis.

the hydrogens at δ_H 3.96 and 3.86 with the carbons at δ_C 138.4 (C-2) and δ_C 147.8 (C-3), respectively.

The absolute configuration of C-6a was assigned as *S*, because it has a negative Cotton effect at 280 nm and a positive Cotton effect at 240 nm in CD curve.[17] Additionally, this was confirmed by the positive value of optical rotation $[\alpha]_D^{25}$ = +51.7 (*c* 0.38, $CHCl_3$).[18] Therefore, alkaloid **1** was determined as (*S)*-3-methoxy-nordomesticine, an aporphine alkaloid reported previously from *Nectandra sinuata*[19] also belonging to the Lauraceae family. This report corrects and completes spectroscopic data for this compound.

Alkaloid **2** was obtained as a yellow oil that gives a positive reaction to Dragendorff reagent and its optical rotation value was $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ = +33.3 (*c* 0.60, $CHCl_3$). The UV and IR spectrum of **2** were similar to those of **1**, except for the appearance, in both spectra, of absorptions due to a carbamate group at 282 nm and at 1688 cm^{-1}, respectively.[20] The ^{1}H and ^{13}C NMR spectra showed a similar profile to **1**. In the ^{1}H NMR two new signals appeared at δ_H 4.29 (2H, *m*) and 1.29 (3H, *m*), as well as the displacement of the signal H-5, due to the presence of a deprotecting group in proximity. The COSY experiment showed the correlation of the signals δ_H 4.29 and 1.29, which indicates the presence of an ethyl group that due to its displacement, suggested to be attached to a heteroatom. The ^{13}C NMR and DEPT spectra showed the appearance of three new signals at δ_C 158.8 (C), 61.0 (CH_2), and 14.8 (CH_3), typical signals of an ethoxycarbonyl group attached to a nitrogen atom. The absolute configuration of C-6a was determined as *S,* because it showed the same Cotton effects as **1**. Therefore the alkaloid compound **2** was identified as (*S*)-*N*-ethoxycarbonyl-3-methoxy-nordomesticine. Its ESIMS spectrum gave a pseudomolecular ion peak at *m/z* 414 $[M+H]^+$ corresponding to the molecular formula of $C_{22}H_{22}NO_7$, and fragmentations were due to the loss of CH_3OH and CH_3CH_2OH at *m/z* 382 $[M+H-32]^+$ and *m/z* 368 $[M+H-46]^+$, respectively. The negative ion mode ESI spectrum showed peaks at *m/z* 412 $[M-H]^-$ and *m/z* 383 $[M-H-29]^-$, being the last one the loss of an ethyl group. This type of alkaloids with substituents *N*-ethoxycarbonyl have been isolated from *Lindera angustifolia* (Lauraceae).[21]

Alkaloid **3,** was obtained as a yellow oil, with an optical rotation value of $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ = +7.5 (*c* 0.53 $CHCl_3$). The IR spectrum was similar to that of alkaloids **1** and **2.** Additionaly, there were observed the presence of absorptions at 2830, 2700, 1739 cm^{-1}, characteristic for formyl group. In HRESIMS negative mode was observed the pseudomolecular ion $[M-H]^-$ at *m/z* 369.1133, corresponding to the molecular formula of $C_{20}H_{20}NO_6$. The negative ion mode of ESI-MS spectra showed the loss of a formyl group at *m/z* 339 $[M-H-29]^-$. The

NMR profile was similar to those of the alkaloids **1** and **2**. In the ^{1}H NMR appeared different signals belonging to a mixture of two isomers in 3:1 ratio. The comparison of the major isomer with alkaloid **1** in NMR, showed the same pattern of substitution of aromatic ring, besides the appearance of two new signals at δ_H 8.25 (1H, *s*) in the ^{1}H and at δ_C 161.9 in the ^{13}C of the formyl group to **3**. This functionality on the nitrogen led to formation of rotational isomers, which have been described previously for alkaloids with a *N*-formyl and *N*-acetyl group.[22] The absolute configuration was determined as *S,* in the same manner as that for **1** and **2.** Therefore, this alkaloid **3** was identified as (*S*)-*N*-formyl-3-methoxy-nordomesticine.

Alkaloid **4** was obtained as a white solid with a melting point of 222-223 °C (MeOH), and with an optical rotation of $[\alpha]_D^{25}[\alpha]_D^{25}$= +47.0 (*c* 0.55 $CHCl_3$). The analysis of NMR data of **4** revealed that it has the same basic skeleton as **2**, but it has a methyl ester according to the signals at δ_H 3.76 (3H, *s*) and δ_C 52.6 for **4** in place of the signals for ethyl group in **2**. HRESIMS showed a pseudomolecular ion $[M-H]^-$ at *m/z* 398.1233 corresponding to the molecular formula $C_{22}H_{22}NO_7$. Therefore, the alkaloid **4** was identified as (*S*)-*N*-methoxycarbonyl-3-methoxy-nordomesticine.

The antifungal activity was evaluated through the disc diffusion method against *Fusarium oxysporum* f. sp. *lycopersici*,[23] a phytopathogenic fungus that affects tomato crops, causing huge losses for farmers. The alkaloid fraction was active against *F. oxysporum* at 250 µg/µL. The inhibitory activity against the growth of the fungi was moderate at 5 µg/µL for (*S*)-3-methoxy-nordomesticine **1,** while the other alkaloids were ineffective, suggesting that the presence of electron withdrawing substituents on nitrogen atom decrease the antifungal activity.

Although few evaluation reports of aporphines antifungal activity, has been found that these are active against species such as *Candida albicans*,[24,25] but inactive against fungal pathogens as *Cladosporium herbarium.*[26] These results are a new evaluation report of antifungal activity of these alkaloids, where it is emphasized that the type of substituents on the nitrogen of aporphines has an effect on the antifungal activity they present.

The antibacterial activity was evaluated by radial diffusion method reported by Lerhrer[27] against two *Gram* (+) standard strains: *Staphylococcus aureus* 6538 and *Enterococcus faecalis* 29212 and three *Gram* (-): *Escherichia coli* 25922 and *Salmonella tiphymurium*, strains MS7953 and 14028s. Alkaloid **1** (2.5 µg) showed antimicrobial activity against the both Gram positive bacteria evaluated with values of 30 AU as shown Table 2.

It has been reported for the racemic mixture of compound 3-methoxy-nordomesticine, its activity against *E. coli* (MIC= 256 µg/mL) and *S. aureus* (MIC= 512 µg/mL),[28] however in this case the compound **1** is not active against *E. coli.*

Similarly as in the antifungal activity, the results show that the nature of the substituent on nitrogen influences the antibacterial activity (Table 2).

EXPERIMENTAL

General procedures

Melting point was determined by using Mel-temp Fisher Johns, Laboratory Device. UV spectra were recorded on a Perkin Elmer Lambda 2S and CD spectra on a JASCO J-720 spectrometer. IR spectra were obtained on Perkin Elmer FT-IR Panagon 500 series 1000 as a thin film. Optical rotations were recorded on Schmidt-Haensch polarimeter. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra, as well as 2D spectra (COSY, HMQC, and HMBC) were performed on a Bruker Avance 400 MHz spectrometer using $CDCl_3$ as an internal reference. HRMS were determined on a Shimadzu LCMS-IT-TOF mass spectrometer system with ESI in positive ion mode and negative ion mode. Column chromatography (CC) was carried out with silica gel (70-230 and 230-400 mesh, Merck), and analytical chromatography was performed using silica gel 60 PF_{254} (0.25 mm).

Plant material

The stems of *O. macrophylla* were collected in July 2006 from Nocaima, Colombia by W. Delgado. The botanical specimen was identified by A. Jara, and a voucher specimen (COL-517191), and was deposited in the Colombian National Herbarium of the National University of Colombia, Bogotá, Colombia.

Extraction and isolation

Dried and powered stems (2.0 kg) of *Ocotea macrophylla* were extracted with EtOH by maceration at room temperature and concentrated in vacuum to obtain an extract (102.6 g). A sample (48.9 g) of this extract was solubilized in water using ultrasound and acidified with 5% HCl to pH 2.0. The acidic suspension was then filtered and basified with 20% NaOH to pH 8.0, and successively extracted with chloroform. The chloroform fraction (3.01 g) was subjected to column chromatography with silica gel and eluted with a gradient of petroleum ether:AcOiPr (4:6 to 0:10) and AcOiPr:MeOH (10:0 to 0:10), obtaining four fractions (I-IV). Fraction I (347 mg) was chromatographed repetitively by column (CC) on silica gel and eluted with *n*-hexane:AcOiPr (9:1-8:2), $CHCl_3$, and Tol:AcOiPr 7:3. This yielded alkaloids **1** (7 mg) and **2** (4 mg). Fraction II (250 mg) was submitted to column chromatography on silica gel and eluted with $CHCl_3$:AcOEt (85:15), then submitted to CC on Sephadex LH-20 (CH_3OH) to yield alkaloid **3** (8 mg). Fraction IV (1433 mg) was purified by repetitive column chromatography on silica gel using CH_2Cl_2:MeOH 97:3 and CH_2Cl_2 as elution systems. Finally, successive washings with MeOH yielded alkaloid **4** (98 mg).

(S)-3-Methoxy-nordomesticine (**1**): Yellow oil; $[\alpha]_D^{25}[\alpha]_D^{25}$= +51.7 (*c* 0.38, $CHCl_3$); UV (MeOH) 224, 308 nm; IR (film): ν_{max} 3404,

Table 2. Results antibacterial activity of compounds isolated

Muestra	Cantidad (µg)	29212		6538		25922		14028s		MS7953		EG10627	
		mm	Ua	mm	Ua	mm	Ua	mm	Ua	mm	Ua	mm	Ua
Ampicilllin	2.54	-	-	-	-	-	-	-	-	-	-	-	-
Kanamycin	6.25	6	60	6	60	5	50	6	60	7	70	-	-
Tetracycline	2.89	7.5	75	7	70	8	80	8	80	5	50	-	-
DMSO	100%	-	-	-	-	-	-	-	-	-	-	-	-
1	2.5	3	30	3	30	-	-	-	-	-	-	-	-
2	2.5	-	-	-	-	-	-	-	-	-	-	-	-
3	2.5	-	-	-	-	-	-	-	-	-	-	-	-
4	2.5	-	-	-	-	-	-	-	-	-	-	-	-

2923, 2855, 1585, 1464, 1274, 1129, 1041, 935, 757 cm^{-1}; CD $[\theta]_{242}$ +3345, $[\theta]_{280}$ -250; for ^1H and ^{13}C NMR spectroscopy data, see Table 1; negative ESIMS: *m/z* = 340 $[M-H]^-$; HR-ESIMS: *m/z* = 340.1178 $[M-H]^-$ (calcd. 340.1185 for $C_{19}H_{19}NO_5$).

(S)-N-Ethoxycarbonyl-3-methoxy-nordomesticine (**2**): Yellow oil; $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ = +33.3 (*c* 0.60, $CHCl_3$); UV (MeOH) 220, 282, 308 nm; IR (film): ν_{max} 3402, 2928, 2830, 2700, 1739, 1464, 1236, 1145, 1039, 936, 757 cm^{-1}; CD$[\theta]_{238}$ +3634, $[\theta]_{280}$ -100; for ^1H and ^{13}C NMR spectroscopy data, see Table 1; positive ESIMS: *m/z* = 414 $[M+H]^+$, 382 $[M+H-CH_3OH]^+$, 368 $[M+H-CH_3CH_2OH]^+$; negative ESIMS: *m/z* = 412 $[M-H]^-$, 383 $[M-H-CH_2CH_3]^-$; HR-ESIMS: *m/z*= 412.1385 $[M-H]^-$ (calcd. 412.1396 for $C_{22}H_{22}NO_7$).

(S)-N-Formyl-3-methoxy-nordomesticine (**3**)**:** Yellow oil; $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ = +7.5 (*c* 0.53, DMSO); UV (MeOH) 220, 282, 310 nm; IR (film): ν_{max} 3403, 2925, 1688, 1464, 1270, 1145, 1039, 936, 768 cm^{-1}; CD $[\theta]_{242}$ +2321, $[\theta]_{280}$ -250; for ^1H and ^{13}C NMR spectroscopy data, see Table 1; positive ESIMS: m/z = 369 $[M+H]^+$; negative ESIMS: *m/z* = 368 $[M-H]^-$, 339 $[M-H-CHO]^-$; HR-ESIMS: *m/z*= 369.1133 $[M-H]^-$ (calcd. 369.1134 for $C_{20}H_{18}NO_6$).

(S)-N-Methoxycarbonyl-3-methoxy-nordomesticine (**4**): Solid cream, melting point 222-223ºC (MeOH); $[\alpha]_D^{25}$ $[\alpha]_D^{25}$ = +47.0 (*c* 0.55, $CHCl_3$); UV (MeOH) 220, 282, 308 nm; IR (film): ν_{max} 3372, 2927, 1685, 1466, 1272, 1146, 1039, 935, 876 cm^{-1}; CD $[\theta]_{239}$ +303 $[\theta]_{280}$ -10; for ^1H and ^{13}C NMR spectroscopy data, see Table 1; positive ESIMS: *m/z* = 400 $[M+H]^+$, 368 $[M+H-CH_3OH]^+$; negative ESIMS: *m/z* = 398 $[M-H]^-$, 339 $[M-H-CH_3OCO]^-$; HR-ESIMS: *m/z*= 398.1233 $[M-H]^-$ (calcd. 398.1240 for $C_{21}H_{20}NO_7$).

Antifungal assay

F. oxysporum was obtained from the culture collection of University of Cundinamarca (Department of Agronomy, Laboratory of Phytopathology). PDA was used as medium for antifungal activity assays. The culture medium was inoculated with 100 µL of a solution of 10^5 spores. The samples were prepared in solutions of different concentrations, corresponding to 50, 25, and 10 µg/µL of the alkaloid fraction and 5, 2.5 1.0, 0.5, 0.2, and 0.1 µg/µL of the pure alkaloids. Ten microliters of samples were applied on the filter paper discs and placed on the inoculated medium. The plates were sealed and left in an incubator for 3 days at 25 °C. Clear zones appearing against a growing fungus indicated the minimal amount of fraction or alkaloid required to inhibit the fungi growth. Three replicates were made for each treatment. Benomyl (benzimidazole – 5 µg) was used as a positive control*,* and acetone served as a negative control.[23]

Antibacterial assays

The antibacterial activity was evaluated by radial diffusion method adapted from the methodology previously published by Lehrer.[27] The compounds were evaluated against two Gram (+) strains: *Staphylococcus aureus* ATCC 6538 and *Streptococcus fecalis* ATCC 29212 and three Gram (-) strains: *Escherichia coli* ATCC 25922 and *Salmonella tiphymurium*, ATCC 14028s and *Salmonella tiphymurium* MS7953. A colony isolated from each strain, was deposited in 3 mL of soy trypticase (TSB) for Gram (+) strains and Luria Broth (LB) for Gram (-) strains, and were incubated at 37 °C with stirring, until the microorganisms were in the logarithmic phase. The supernatant was removed and the sediment obtained was re-suspended in phosphate buffer (PBS), followed by washes with PBS and centrifuging. Finally the sediment was re-suspended in PBS and the optical density determined in 620 nm to calculate the number of CFU (colony forming units) per milliliter. It disperses a certain volume that contains 4 x 10^7 CFU in each dish. The measured volume was mixed and homogenized in 15 mL agarose fused to more or less 45 °C. This bacterial suspension was served in petri dishes and left to solidify at room temperature, after which were made of 2 mm diameter holes with a sterile punch.

The test samples were prepared dissolving 1 mg of the pure compound in 500 µL of DMSO, which are placed 8 µL of the sample in duplicate and incubated at 37 °C for 30 min. After this time the nutrient medium was added, which contains molten agar agar and TSB, incubated for 18 h at 37 ºC and then diameter of inhibition zones was measured by the activity of the compound. Positive controls used were different antibiotics, Ampicillin (50 mg/mL), Kanamycin (10 mg/mL) and Tetracycline (4.12 mg/mL) at a dilution 1:100 in PBS and was used as negative controls DMSO and PBS, each control 8 µL be served by each well. The diameters of inhibition zones were measured in millimeters and the results were reported as units of activity according to the ratio which stipulates that 1 Unit of Action (UA) is equal to 0.1 mm of the inhibition zone.

SUPPLEMENTARY MATERIAL

1D and 2D NMR spectra for compounds **1**-**4**. This material is available free of charge at http://quimicanova.sbq.org.br, in .PDF format.

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APORPHINE ALKALOIDS FROM *Ocotea macrophylla* (LAURACEAE)

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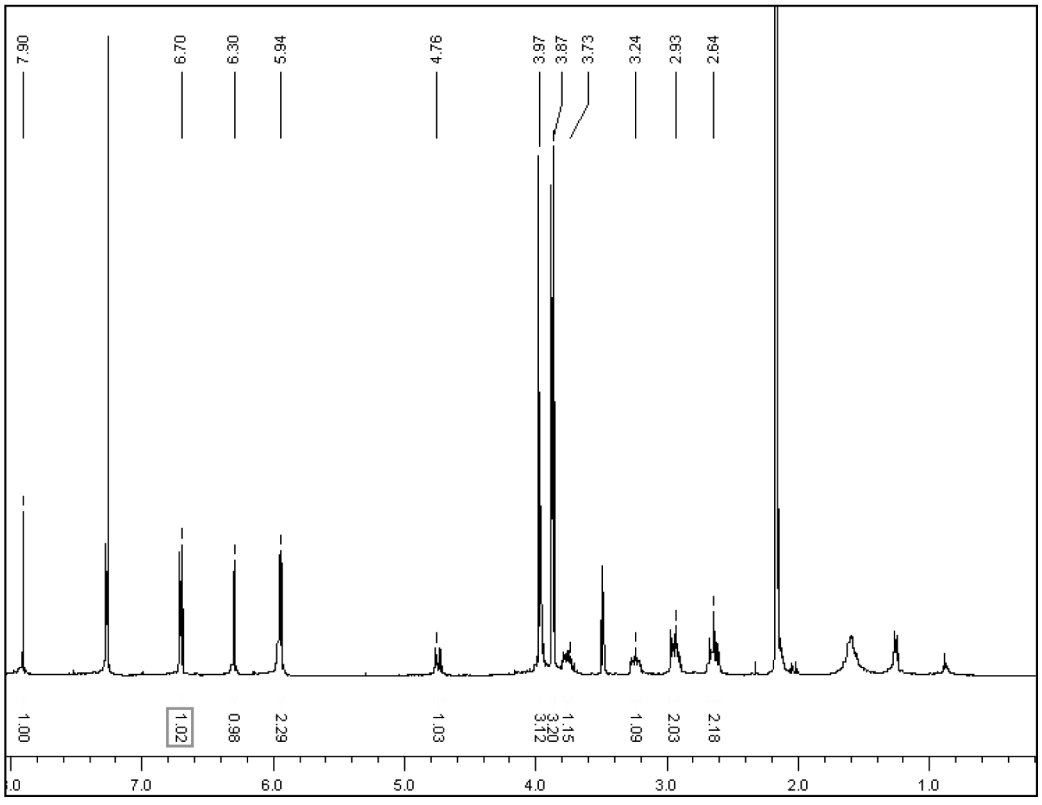

Figure 1S. *The ^{1}H NMR data of* ***1*** *(CDCl3, 400 MHz)*

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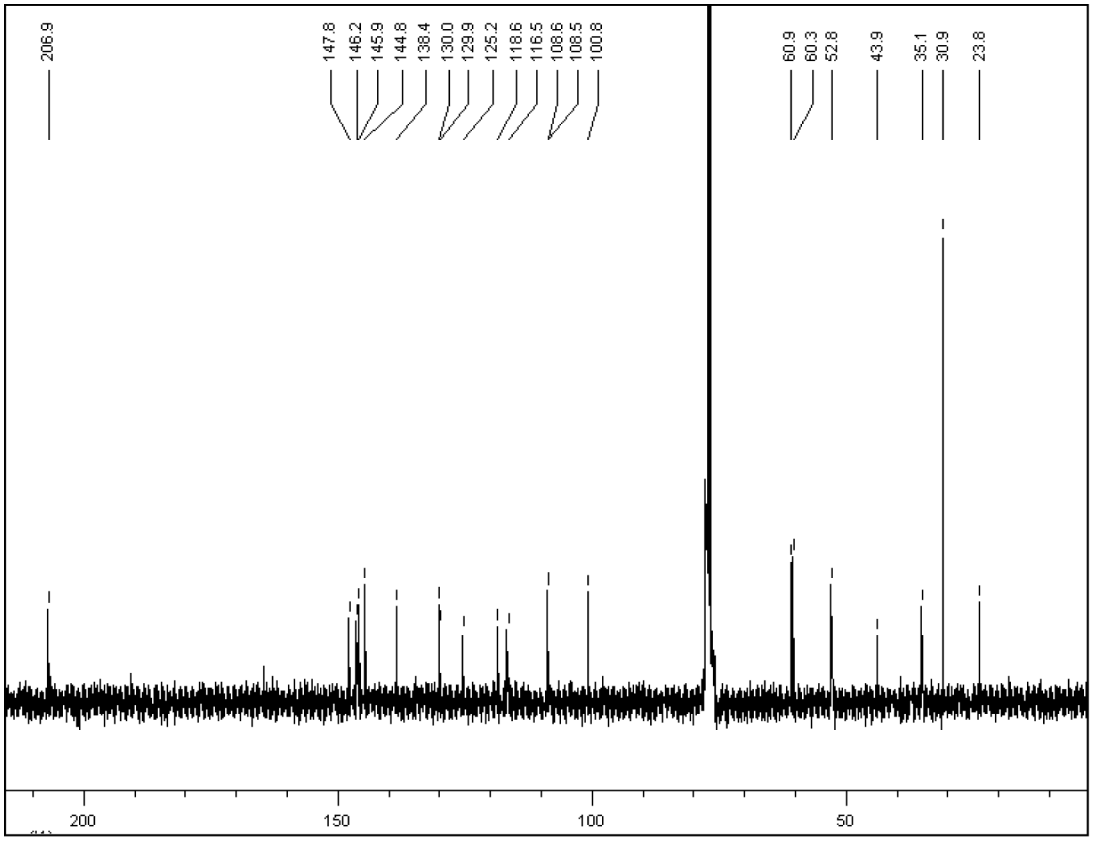

Figure 2S. *The ^{13}C NMR data of* ***1*** *(CDCl3, 100 MHz)*

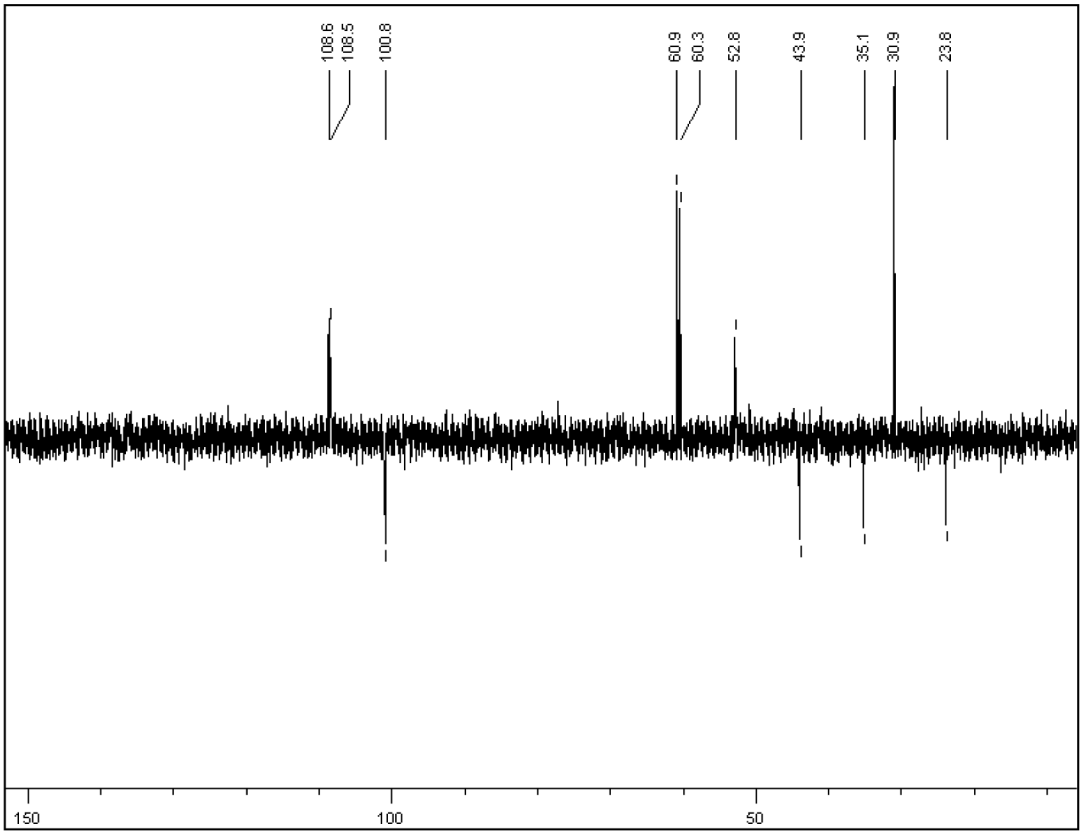

Figure 3S. *The DEPT data of* ***1***

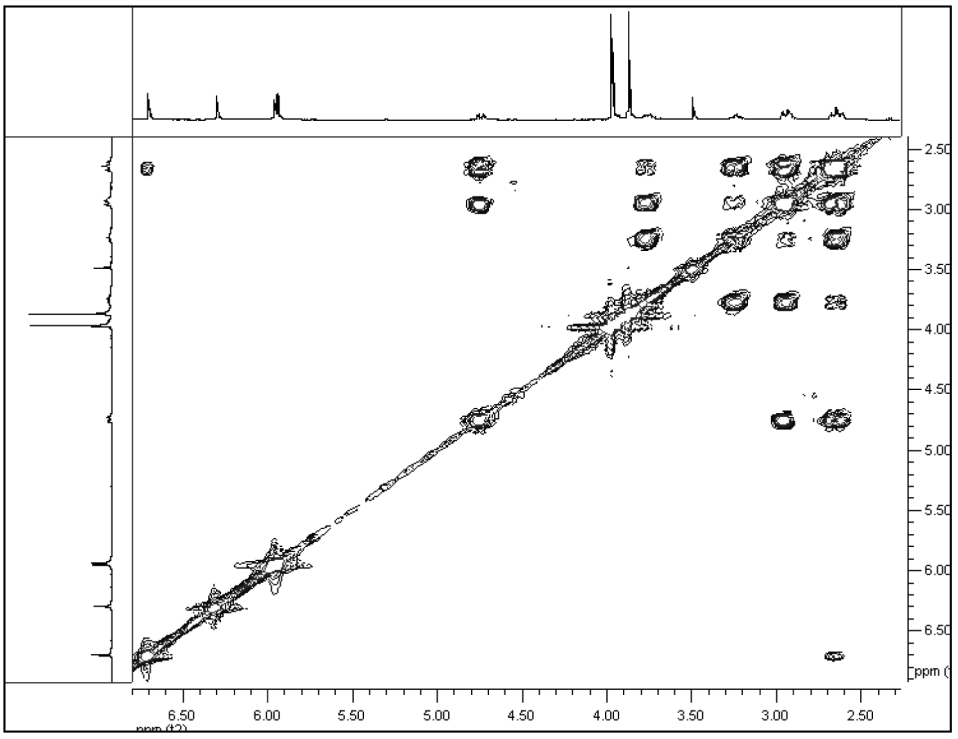

Figure 4S. *The ^{1}H-^{1}H COSY data of* ***1***

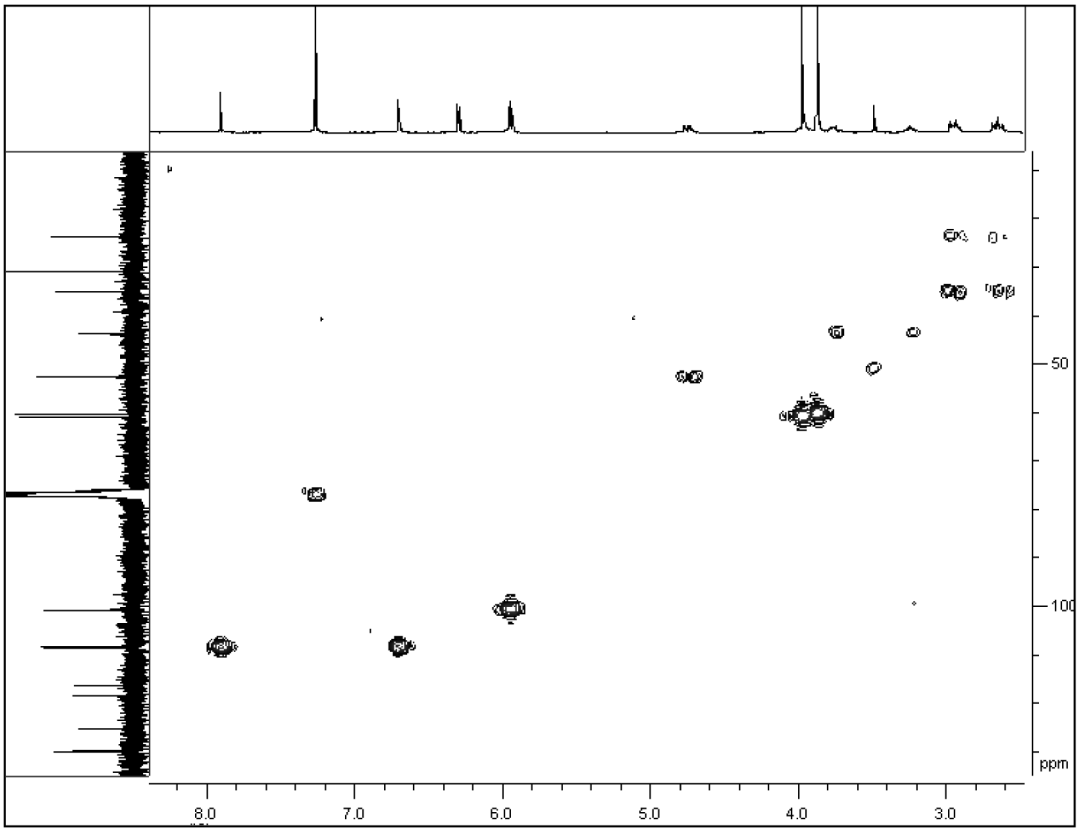

Figure 5S. *The HMQC data of* ***1***

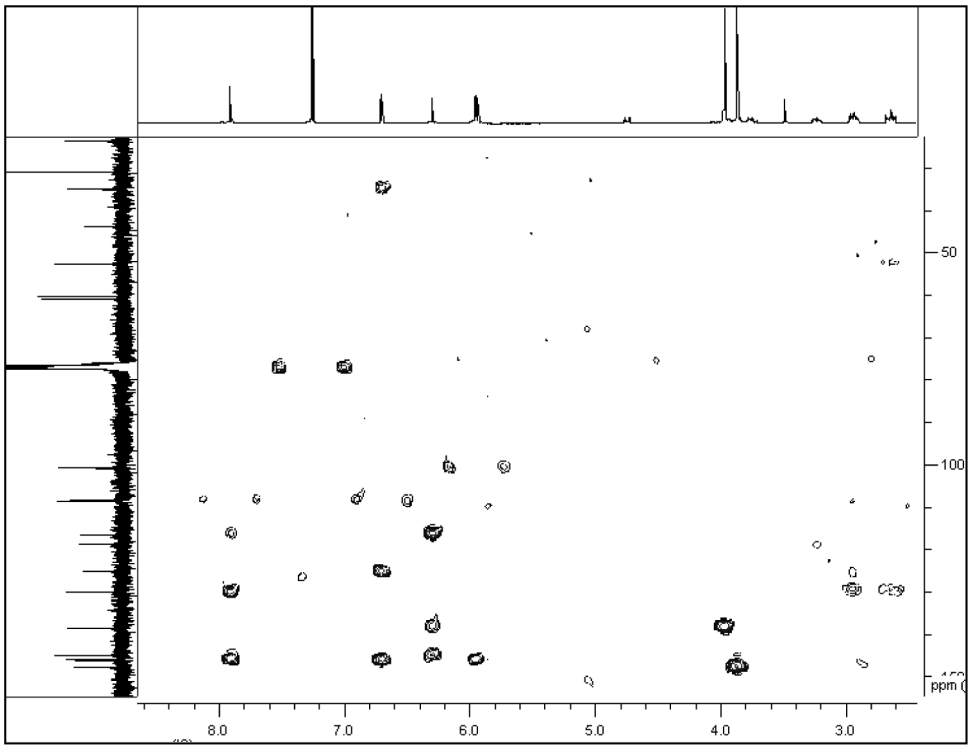

Figure 6S. *The HMBC data of* ***1***

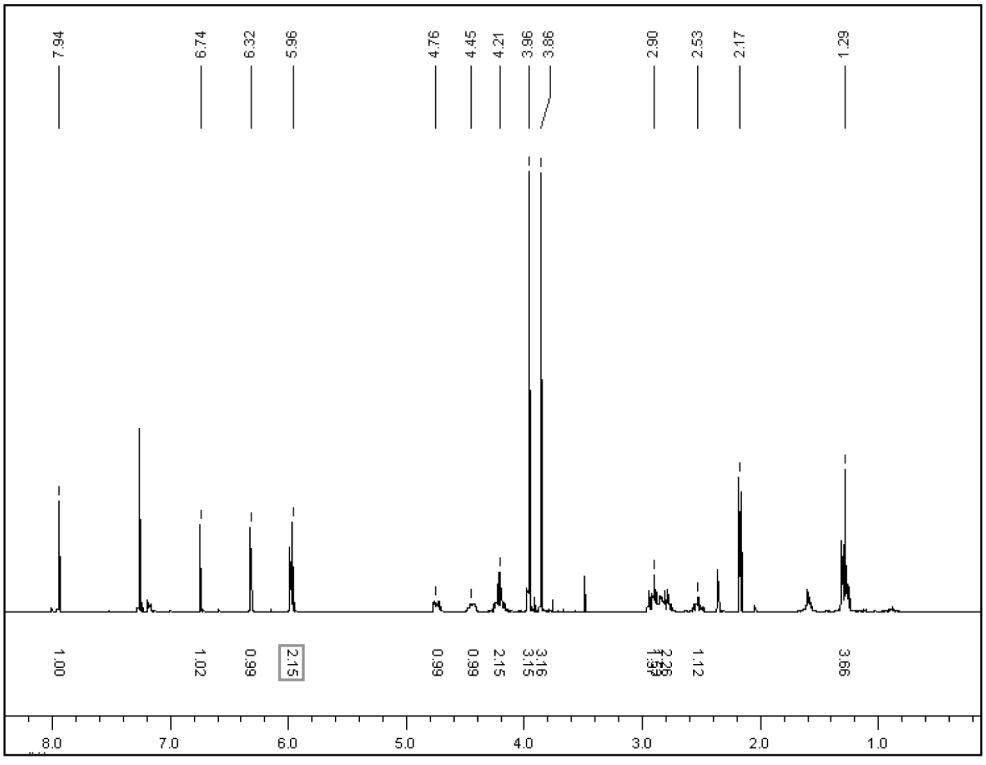

Figure 7S. *The [1]H NMR data of 2 (CDCl3, 400 MHz)*

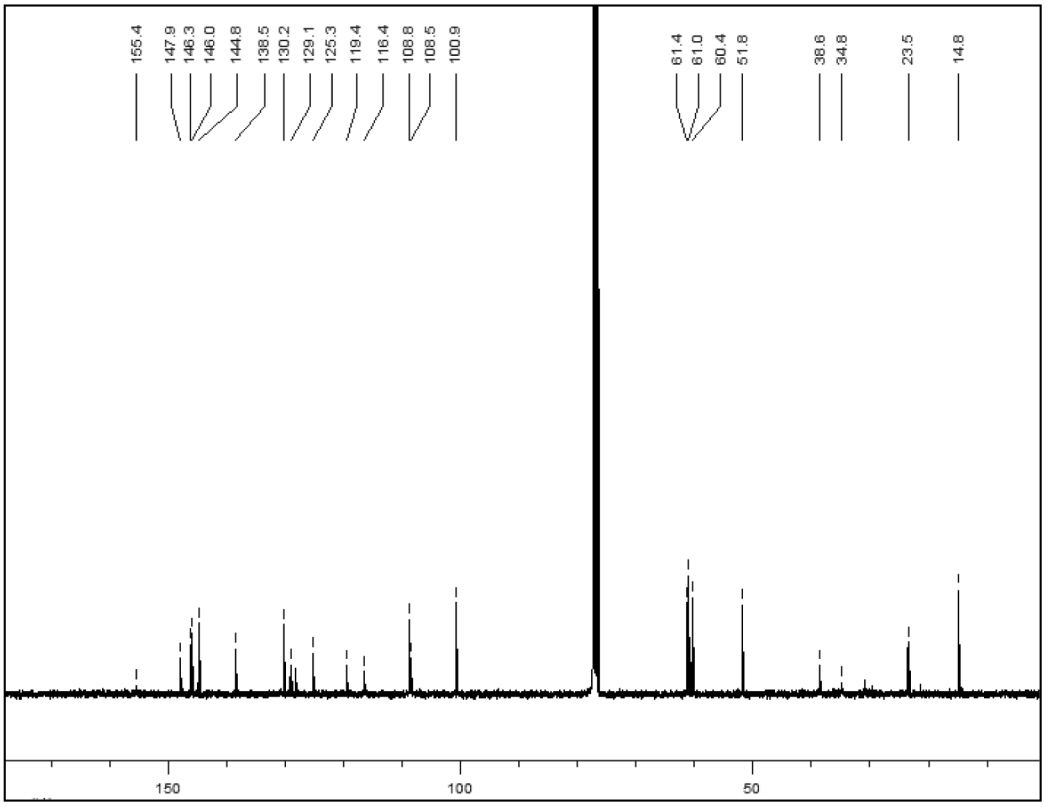

Figure 8S. *The ^{13}C NMR data of* ***2*** *(CDCl3, 100 MHz)*

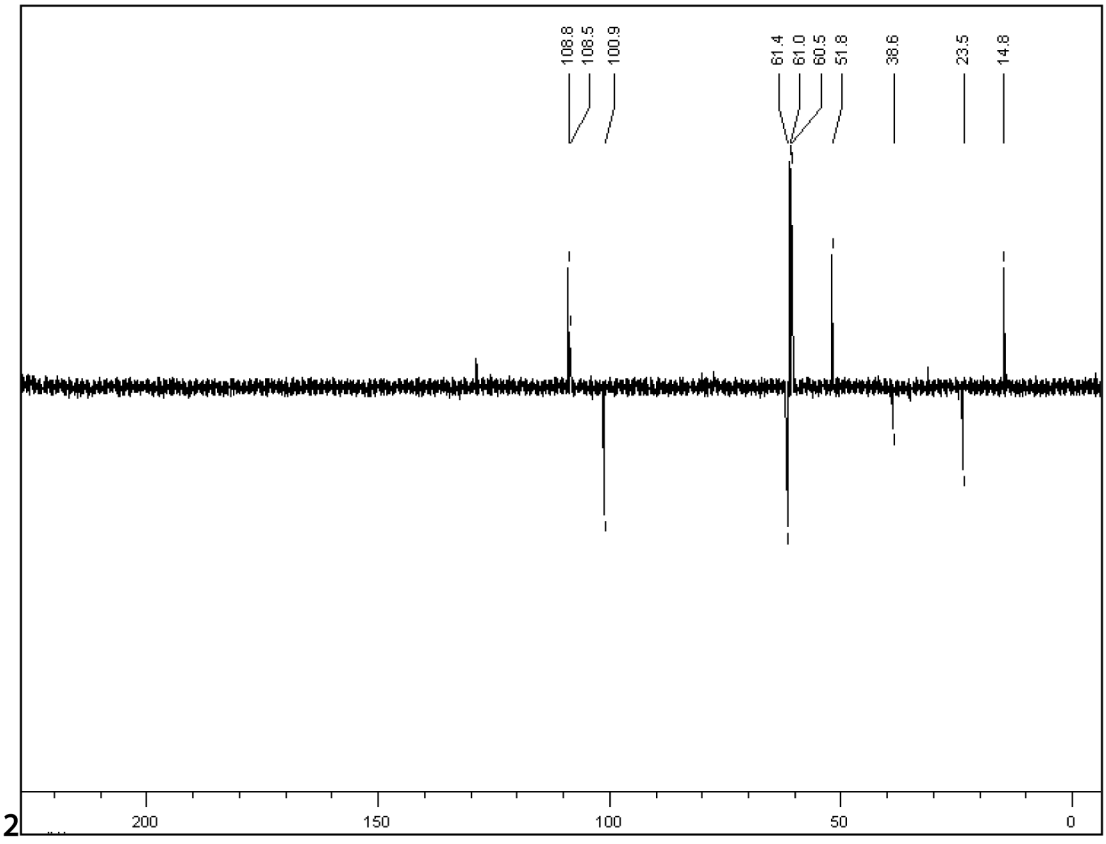

Figure 9S. *The DEPT data of 2*

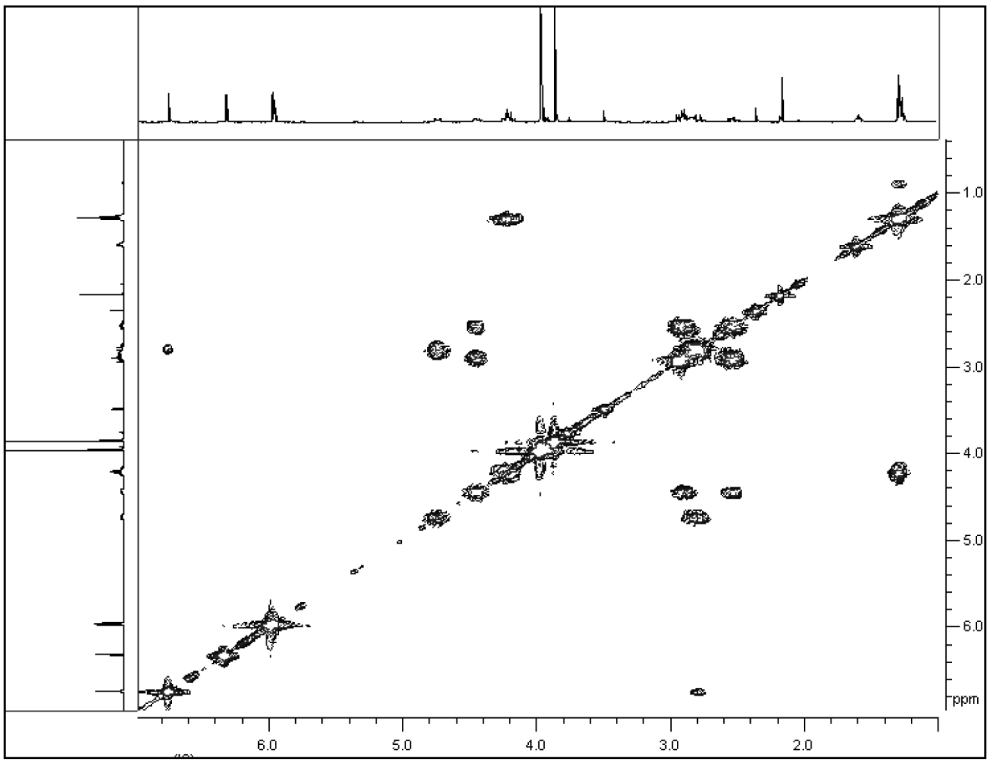

Figure 10S. *The ^{1}H-^{1}H COSY data of* ***2***

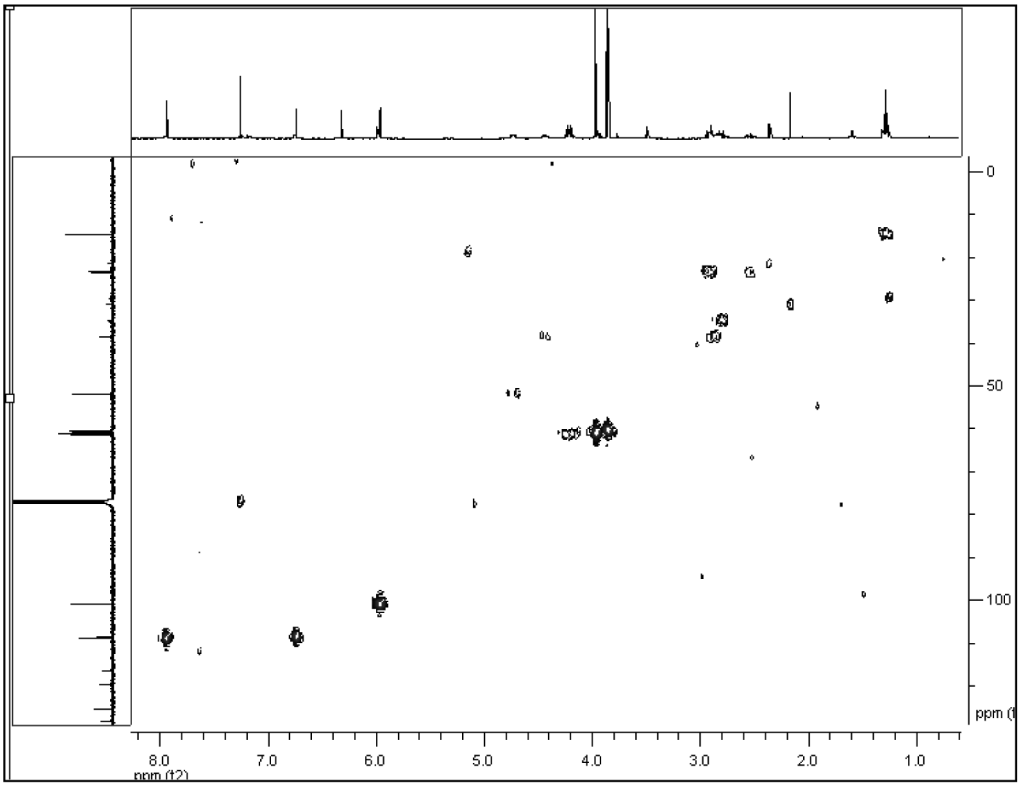

Figure 11S. *The HMQC data of* ***2***

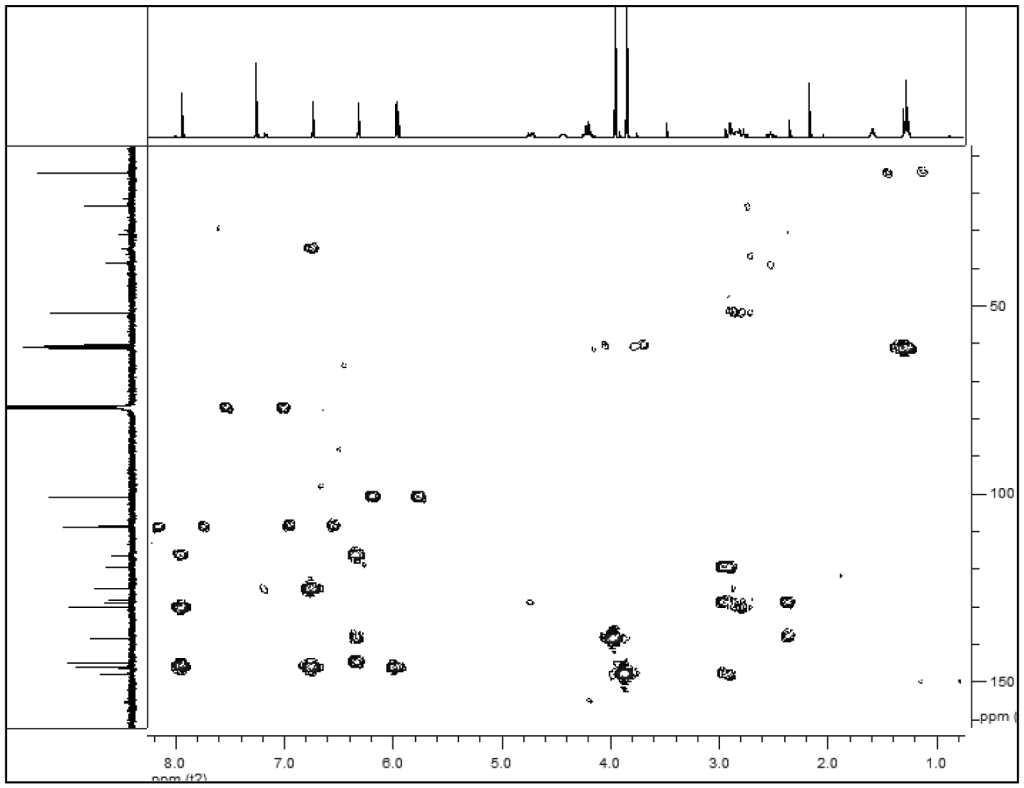

Figure 12S**. The HMBC data of **2

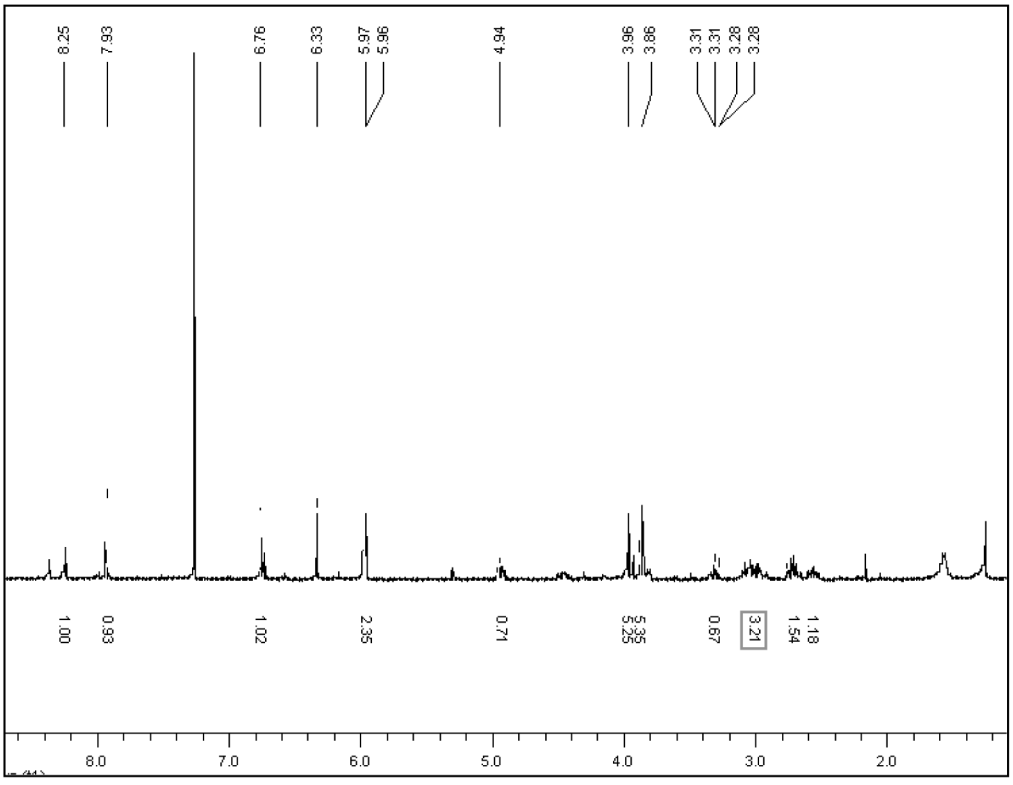

***Figure 13S.** The [1]H NMR data of 3 (CDCl3, 400 MHz)*

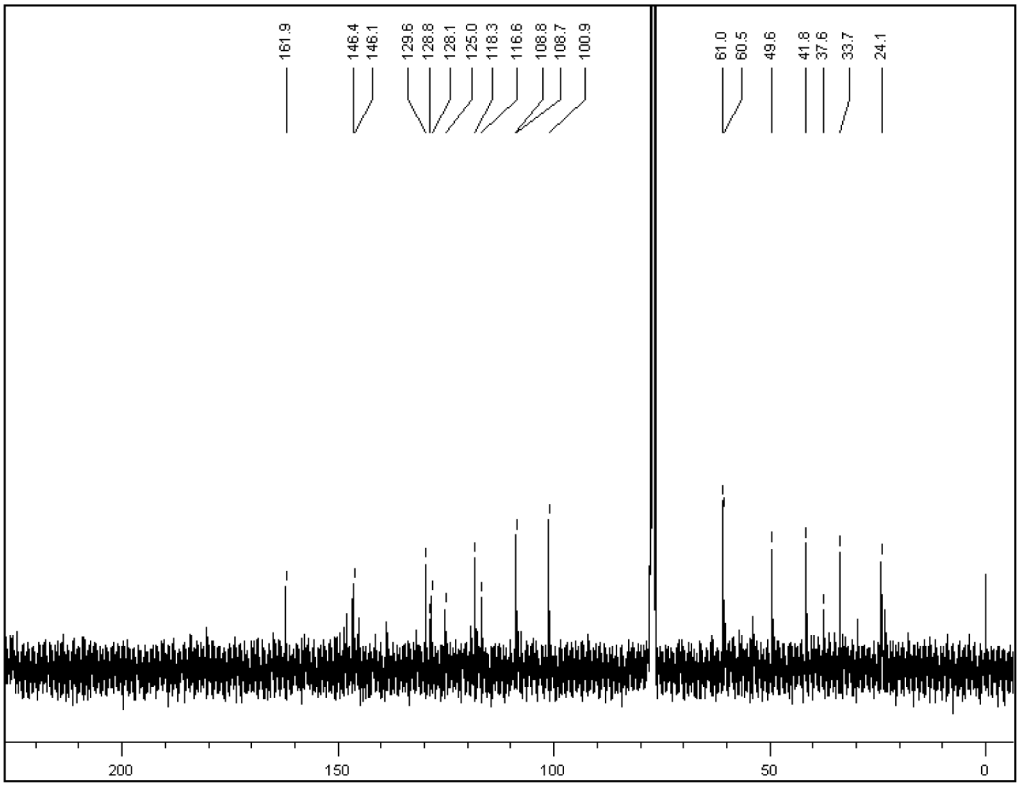

Figure 14S. *The ^{13}C NMR data of **3** (CDCl3, 100 MHz)*

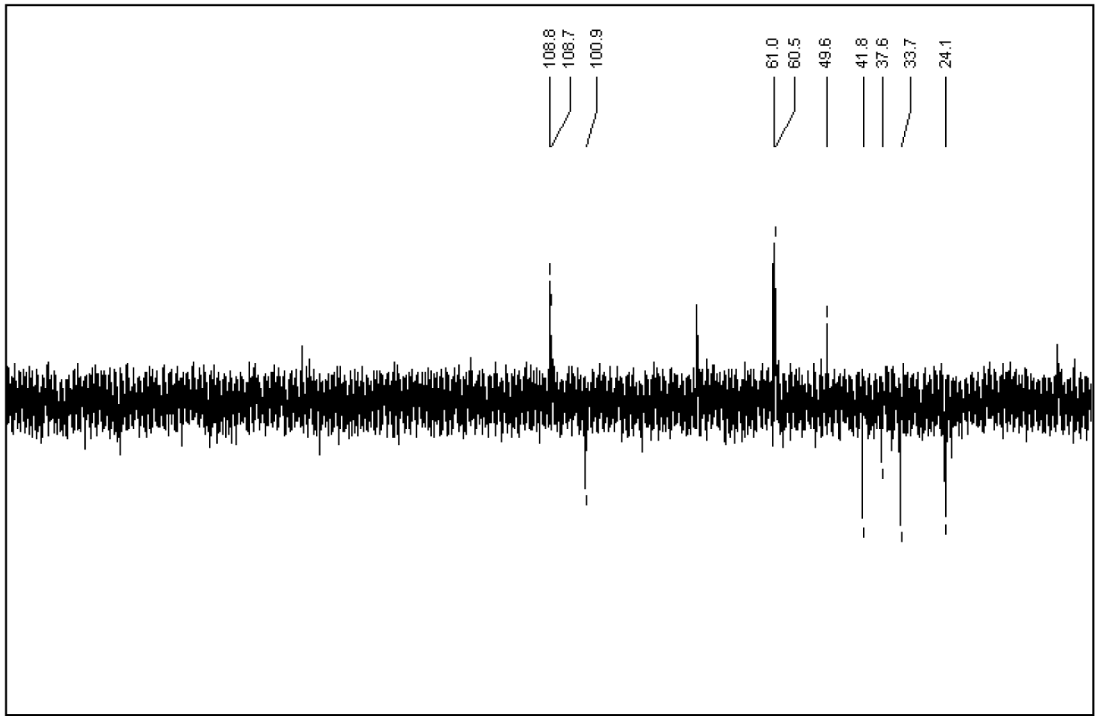

Figure 15S. *The DEPT data of 3*

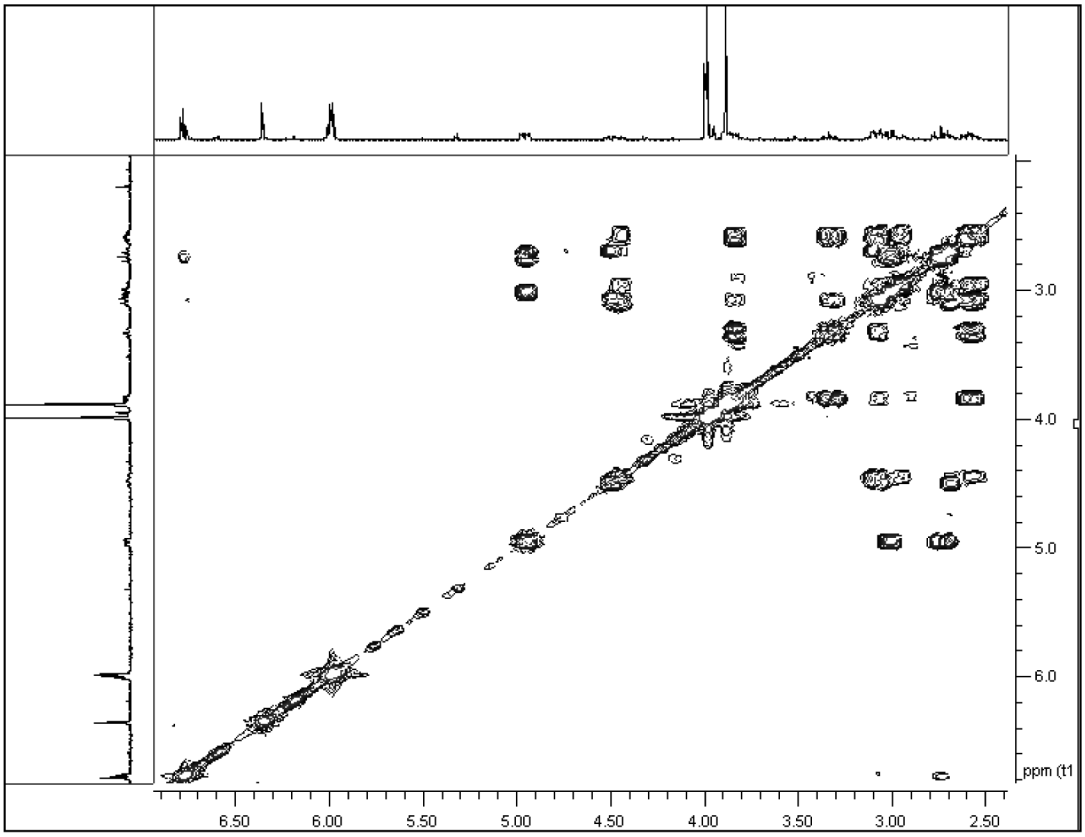

Figure 16S. *The ^{1}H-^{1}H COSY data of 3*

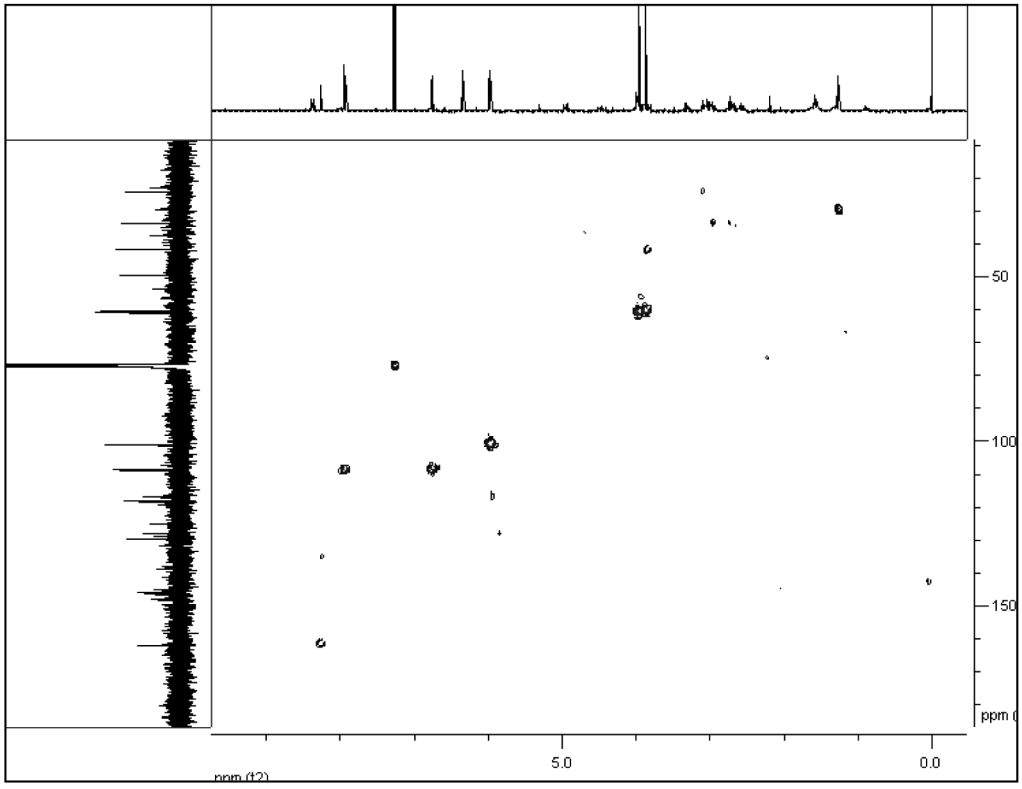

Figure 17S. *The HMQC data of* ***3***

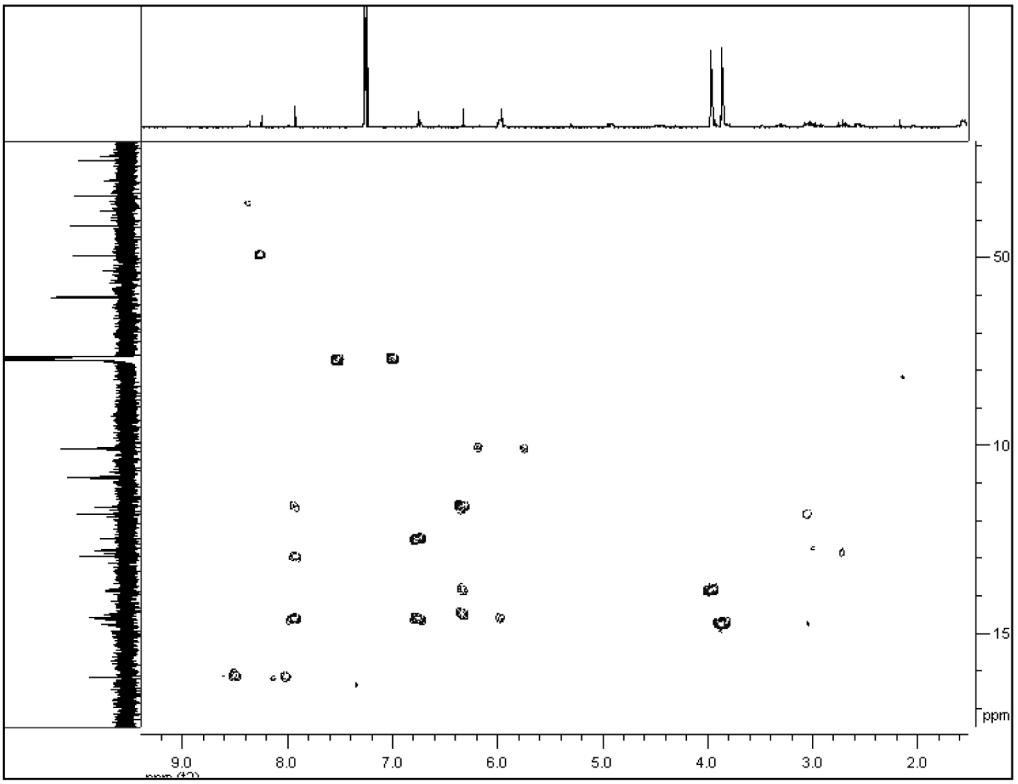

Figure 18S. *The HMBC data of* ***3***

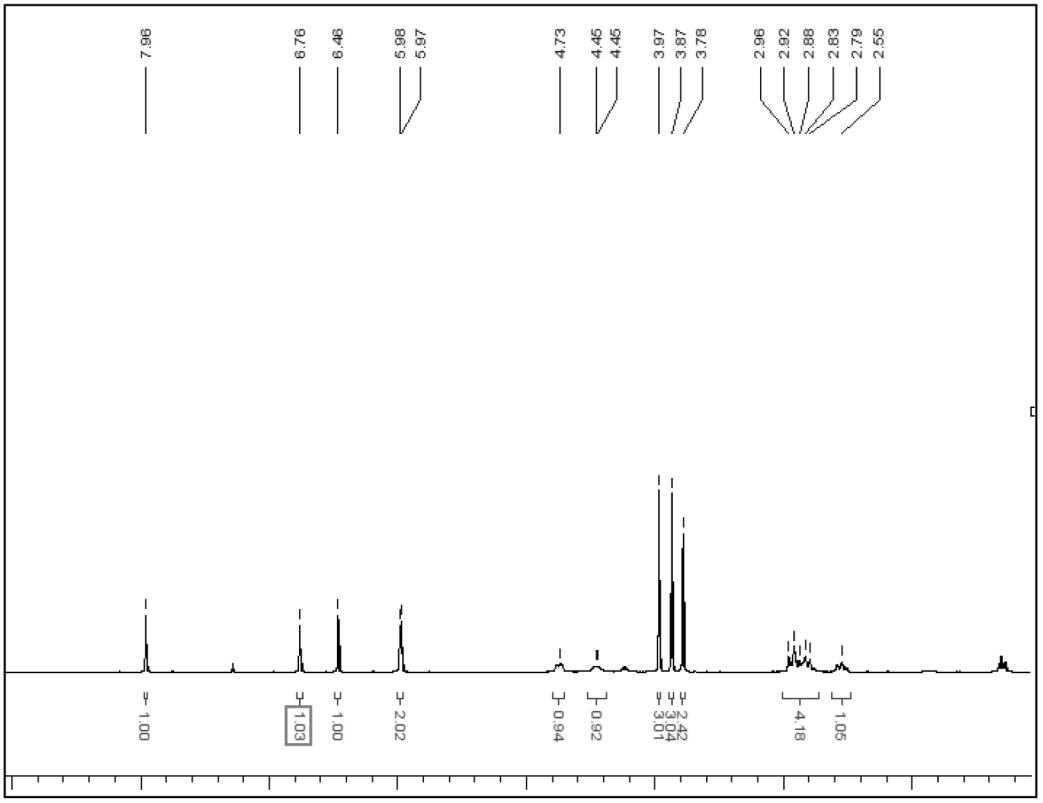

Figure 19S. *The* ^{1}*H NMR data of 4 (CDCl3, 400 MHz)*

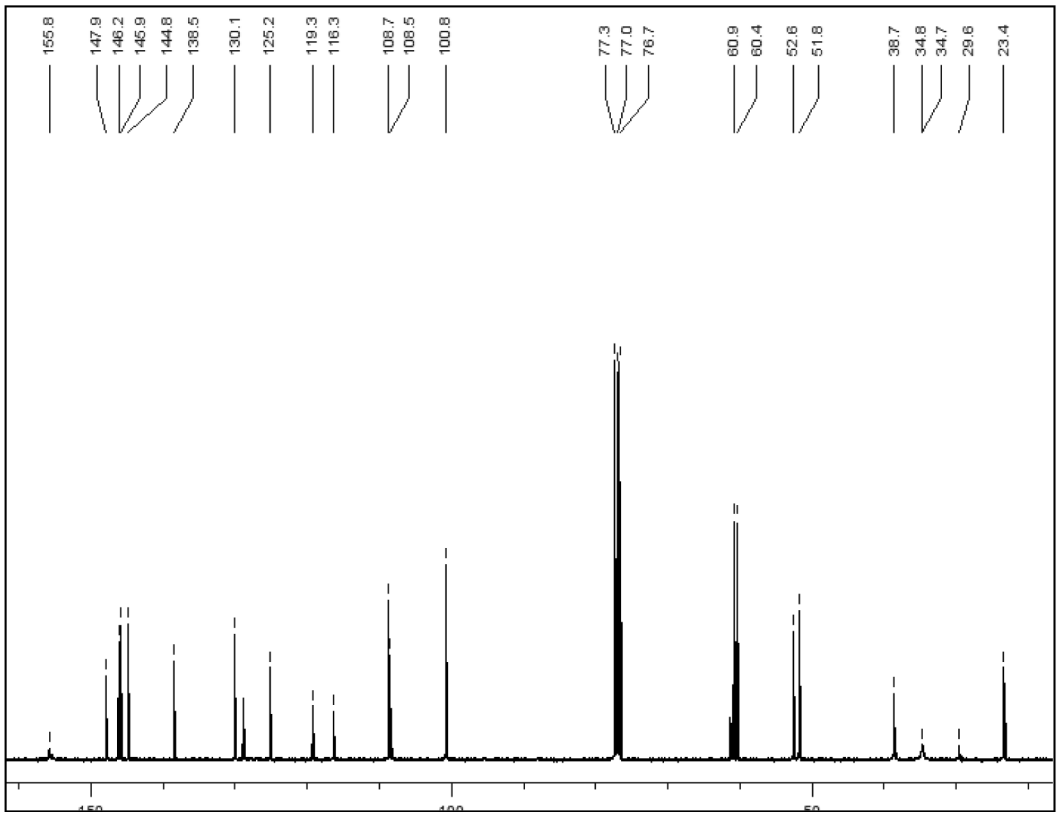

Figure 20S. *The [13]C NMR data of* ***4*** *(CDCl3, 100 MHz)*

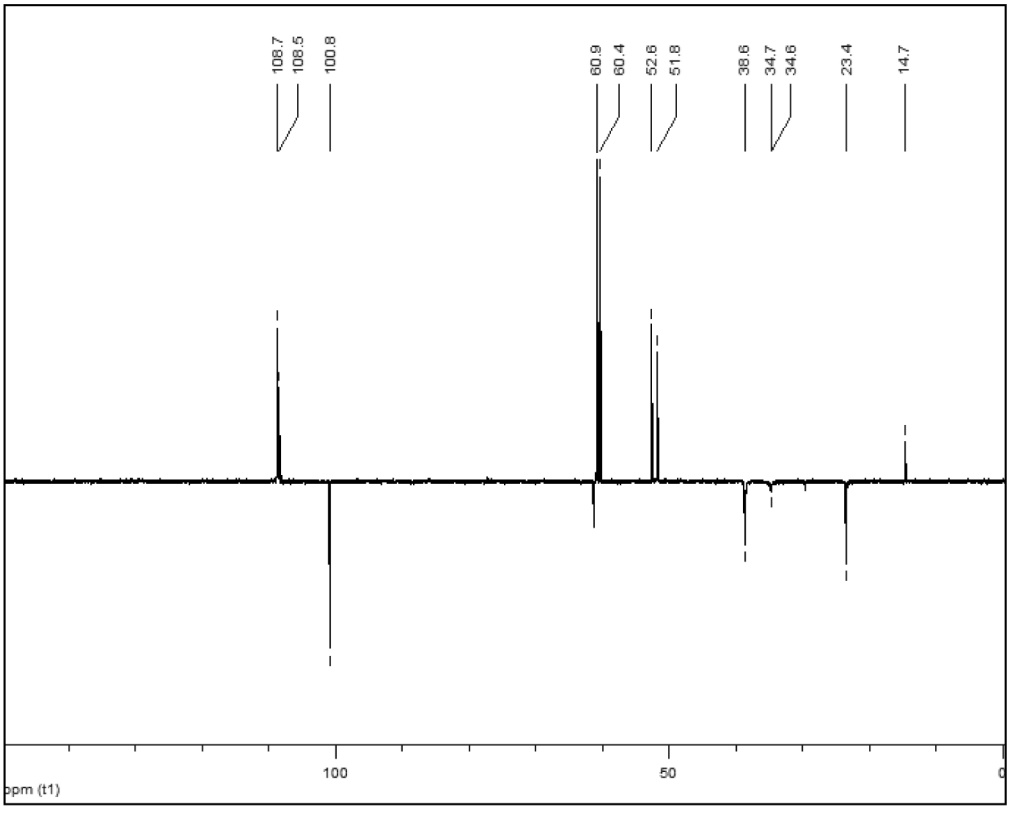

Figure 21S. *The DEPT data of 4*

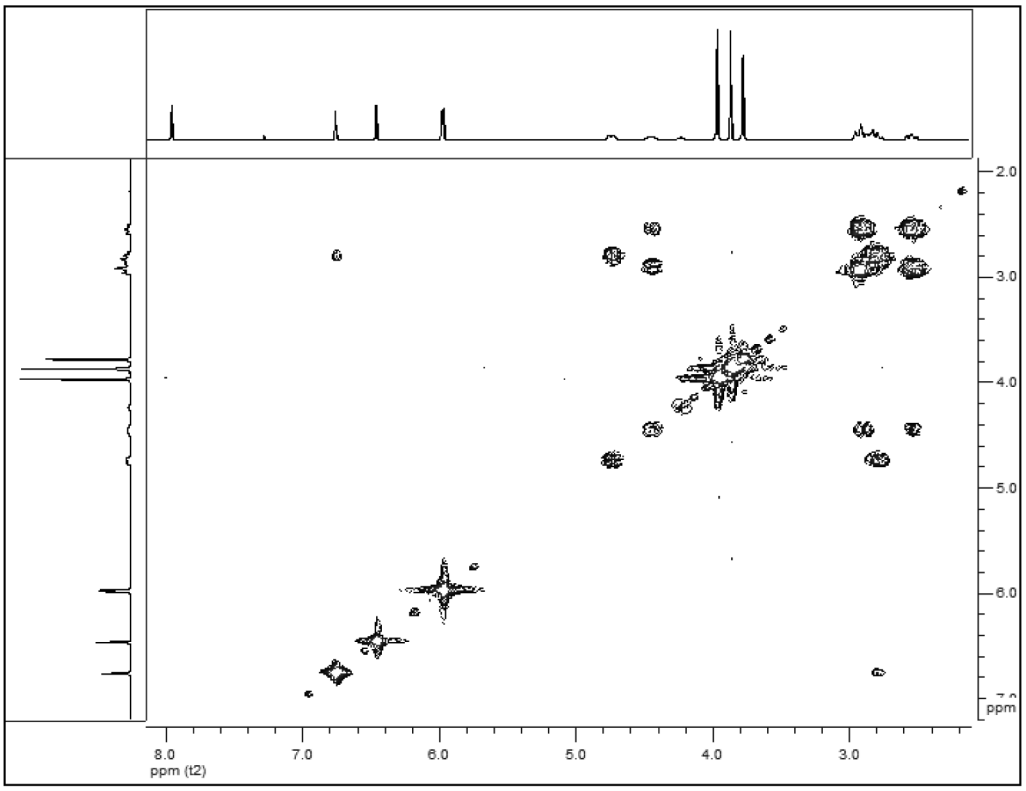

Figure 22S. *The ^{1}H-^{1}H COSY data of 4*

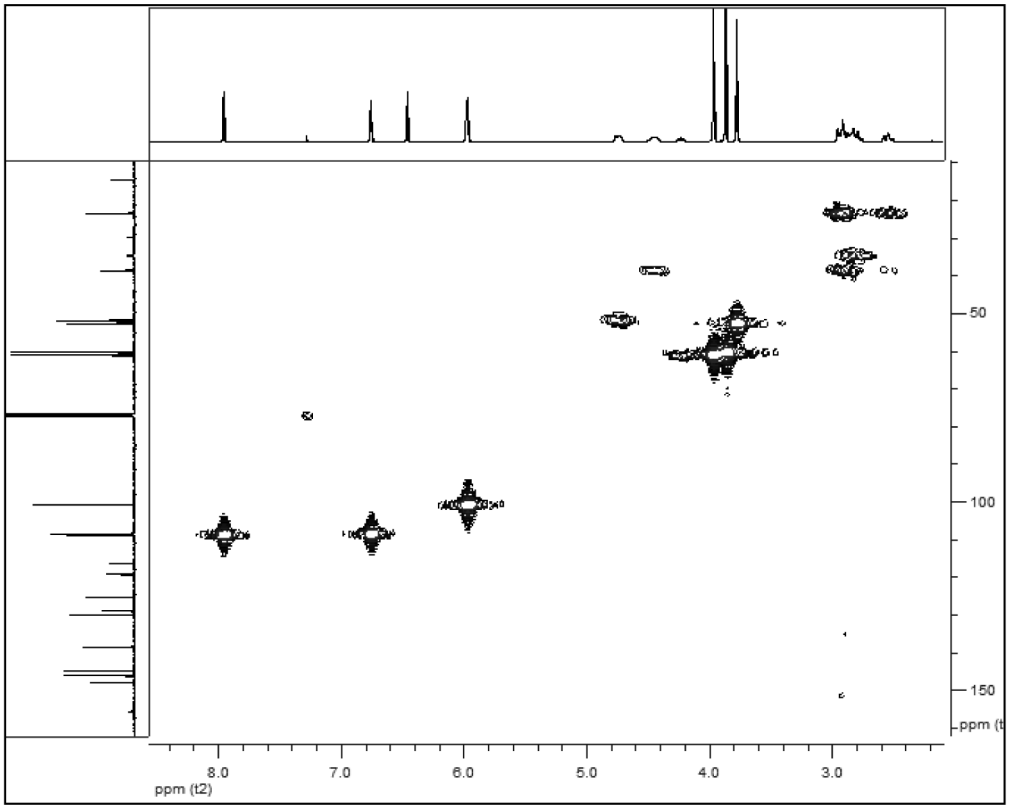

Figure 23S. *The HMQC data of* ***4***

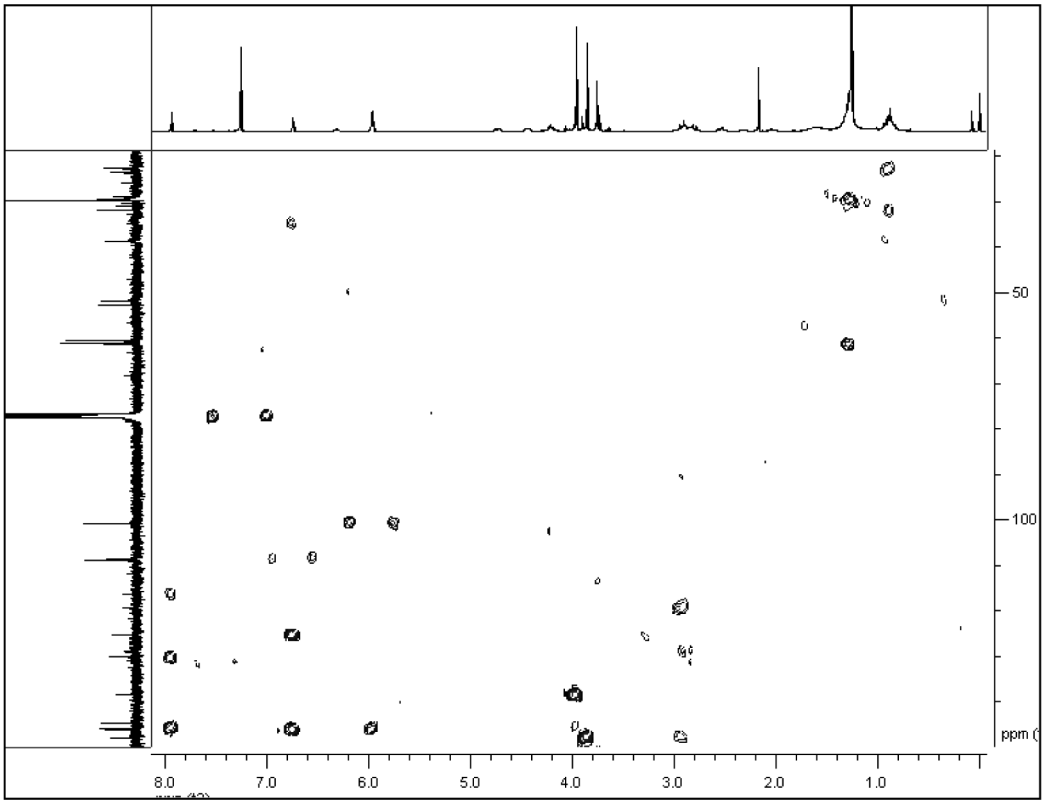

Figure 24S. *The HMBC data of* ***4***