

## Two Novel Eremophilane Sesquiterpenes from an Endophytic Xylariaceous Fungus Isolated from Leaves of *Cupressus lusitanica*

Luciana S. Amaral and Edson Rodrigues-Filho\*

Departamento de Química, Universidade Federal de São Carlos, CP 676,  
13565-905 São Carlos-SP, Brazil

Dois novos sesquiterpenos eremofilanos, cupressolideo A e cupressolideo B, além de dois outros conhecidos, foram isolados a partir do extrato AcOEt do meio de cultura de uma espécie de *Xylaria*, isolada como fungo endofítico dos tecidos saudáveis das folhas de *Cupressus lusitanica*. Estudos espectroscópicos, usando EM e RMN, levaram às estruturas dos dois sesquiterpenos de esqueleto eremofilanos, novos na literatura.

Two new eremophilane sesquiterpenes, cupressolide A and cupressolide B, along with two known sesquiterpenes, has been characterized from the EtOAc extract of a liquid medium where a Xylariaceous fungus, isolated as an endophytic fungus from health tissues of *Cupressus lusitanica* leaves, was cultivated. The structures of the isolated compounds were determined by analyses of their MS and NMR spectroscopic data.

**Keywords:** *Cupressus lusitanica*, *Xylaria*, eremophilane sesquiterpenes, endophytic fungi

### Introduction

*Cupressus lusitanica*, commonly known as a Mexican Cypress and Portuguese Cypress, belongs to the family Cupressaceae and is usually cultivated as an ornamental tree and in commercial forestry plantation.<sup>1,2</sup> Due to its economic importance, this plant was included in our continuous program established to study the chemistry and biochemistry aspects of plant microorganisms interactions, with emphasis on those apparently symbiotic associations.

Among the endophytic fungi isolated from healthy tissues of *C. lusitanica* leaves, we obtained some strains with macro and micro morphology characteristics of those microorganisms belonging to the genus *Xylaria*. Besides these morphologic characteristics, *Xylaria* species are producers of secondary metabolites, including isocoumarin,<sup>3,4</sup> cytochalasins,<sup>4-6</sup> xanthenes,<sup>7</sup> xyloketal,<sup>8-10</sup> sesquiterpenes,<sup>11</sup> that contribute to their classification in this genus. In our study we detected isocoumarins and cytochalasins in the fungus extracts using mass spectrometry, which reinforce the hypothesis of its classification as a Xylariaceous fungus. Among the compounds isolated from the fungus extract, two novel (**1** and **2**) and two known (**3** and **4**) eremophilane sesquiterpenes were identified.

The production of these terpenoids corroborated to classify the fungus within Xylariaceae family. Although it is not clear the importance of these compounds as phytotoxic agents, some members of this class of substances have shown remarkable biological activities, such as anti-inflammatory, antihyperglycemic, cytotoxic, HIV-1 integrase inhibitory.<sup>12-15</sup>

### Results and Discussion

The EtOAc extract of liquid medium from endophytic fungi was chromatographed on silica gel columns to give four compounds (**1-4**). Compounds **3** and **4** were previously reported in the literature.<sup>16,17</sup>

Compound **1** was obtained as a colorless crystal. The IR spectrum displayed a broad band at 3487 cm<sup>-1</sup> characteristic of a hydroxyl group and a band at 1745 cm<sup>-1</sup> attributed to a conjugated  $\gamma$ -lactone. The <sup>13</sup>C NMR spectrum exhibited 15 signals which were assigned, by DEPT 135 and HSQC experiments to three methyls, three methylenes, four methines and three sp<sup>2</sup> carbons, one of this being a carbonyl group. Its ESI-MS spectrum contains an ion peak of [M+H]<sup>+</sup> at *m/z* 265, consistent with the molecular formula C<sub>15</sub>H<sub>20</sub>O<sub>4</sub> which also was in accordance with the NMR data. The <sup>1</sup>H NMR spectrum of **1** showed three signals  $\delta_{\text{H}}$  1.85, 0.83 and 0.96 attributed to CH<sub>3</sub>-13,

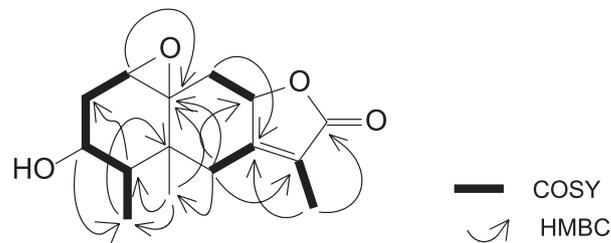
\*e-mail: edson@dq.ufscar.br

CH<sub>3</sub>-14 and CH<sub>3</sub>-15, respectively. <sup>1</sup>H NMR spectrum exhibited the presence of a deshielded signal assignable to an oxymethine hydrogen at  $\delta_{\text{H}}$  3.48 (1H, m, H-3); this signal correlated in COSY spectrum with the  $\delta_{\text{H}}$  1.89 (1H, m, H-2b); 2.44 (1H, ddd, *J* 15.2; 8.4; 4.4 Hz, H-2a); 1.81 (1H, m, H-4). H-4 showed coupling with the methyl group at  $\delta_{\text{H}}$  0.96 (3H, *J* 6.8 Hz, CH<sub>3</sub>-15). The presence of an epoxy group was confirmed by the chemical shifts of H-1 ( $\delta$  3.06), C-1 ( $\delta$  58.5), and C-10 ( $\delta$  63.2).

The COSY spectrum exhibited correlation peaks among the oxymethine hydrogen at  $\delta_{\text{H}}$  4.9 (1H, m, H-8) with hydrogens at  $\delta_{\text{H}}$  2.05 (1H, m, H-9a); 1.82 (1H, m, H-9b); 1.85 (3H, t, *J* 1.2 Hz, CH<sub>3</sub>-13).

HSQC analysis indicated the presence of one tetrasubstituted double bond which was associated with the carbons at  $\delta_{\text{C}}$  159.5; 122.3 (C-7 and C-11, respectively). On the other hand another double bond was observed in the <sup>13</sup>C NMR spectrum, with characteristic chemical shifts of a carbonyl group at  $\delta_{\text{C}}$  174.6 attributed to C-12.

The HSQC spectrum exhibited correlations among the diastereotopic hydrogens at  $\delta_{\text{H}}$  2.23 (1H, br d, *J* 13.6 Hz, H-6b) and  $\delta_{\text{H}}$  2.76 (1H, d, *J* 13.6 Hz, H-6b) with the carbon at  $\delta_{\text{C}}$  35.1. The HMBC spectrum showed coupling of H-6b with C-5, C-7, C-8, C-10, C-11 and CH<sub>3</sub>-14. The COSY and HMBC analysis of **1** led to a partial structure as shown in Figure 1.



**Figure 1.** Selected H-H COSY and HMBC correlations for compound **1**.

The relative stereochemistry of **1** was elucidated using nOe and COSY spectroscopy. The  $\beta$  orientation of H-3 was inferred from the nOe correlation with CH<sub>3</sub>-14 and CH<sub>3</sub>-15. H-8 ( $\delta$  4.90) should be  $\alpha$  oriented as indicated by the homoallylic coupling with the methyl (CH<sub>3</sub>-13,  $\delta$  1.85) bonded to C-11 observed in the COSY experiment. This homoallylic coupling requires a 90° dihedral angle of the methyl group at C-11 with H-6 $\alpha$  and H-8, which was in agreement with the nOe experiment. Furthermore, the nOe spectrum showed correlations of H-6 $\alpha$  with H-4 ( $\delta$  1.81) and of H-6 $\beta$  with CH<sub>3</sub>-13, CH<sub>3</sub>-14 and CH<sub>3</sub>-15. All NMR data are shown in the Table 1.

Compound **2** showed spectroscopic data very similar to those of **1**, indicating the presence of an eremophilane skeleton. The IR spectrum displayed a broad band at 3508 cm<sup>-1</sup> characteristic of a hydroxyl group. The

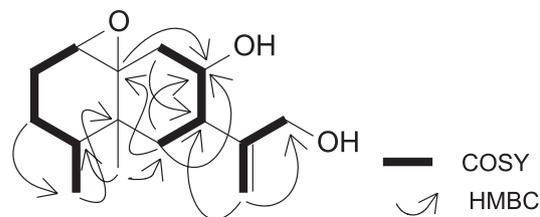
**Table 1.** NMR spectroscopy data for eremophilane sesquiterpene **1**<sup>a</sup>

Position	$\delta_{\text{H}}$ , mult, ( <i>J</i> in Hz)	$\delta_{\text{C}}$	COSY	HMBC (H→C)
1	3.06, d (4.4)	58.5	H2a	C2, C10
2a	2.44, ddd (4.4; 8.4; 15.2)	33.0	H1, H2b, H3	C3, C14
2b	1.89, m		H2a, H3	C1, C10, C3
3	3.48, m	67.4	H2a, H2b, H4	C4, C15
4	1.81, m	40.7	H15, H3	C2, C3
5	-	39.1	-	-
6a	2.23, br d (13.6)	35.1	H6-b, H13, H14	C5, C7, C8, C10, C11, C14
6b	2.76, d (13.6)		H6a	C5, C7, C8, C10, C11, C14
7	-	159.5	-	n.d
8	4.90, m	78.3	H9a, H9b, H13	n.d.
9a	2.05, m	38.0	H9b, H8	C7, C8, C13
9b	1.82, m		H9a, H8	C3, C5, C14, C15
10	-	63.2	-	-
11	-	122.3	-	-
12	-	174.6	-	-
13	1.85, t (1.2)	8.1	H6b, H8, H15	C7, C11, C12, C15
14	0.83, d (1.0)	15.8	H6b	C4, C5, C6, C10, C15
15	0.96, d (6.8)	10.1	H4	C3, C4, C5

<sup>a</sup>The data were acquired at 400 and 100 MHz for <sup>1</sup>H and <sup>13</sup>C respectively in CDCl<sub>3</sub>. TMS was used as internal reference.

$^1\text{H}$  NMR experiment exhibited two olefinic hydrogens, three carbinolic hydrogens and two methyl groups. The EI-MS spectrum of **2** contains an ion peak of  $\text{M}^+$  at  $m/z$  252, consistent with the molecular formula  $\text{C}_{15}\text{H}_{24}\text{O}_3$  and the data observed in the NMR spectrum. The  $^1\text{H}$  NMR spectrum of compound **2** exhibited deshielded signals due the presence of olefinic hydrogens at  $\delta_{\text{H}}$  5.26 (1H, d,  $J$  0.8 Hz, H-12a) and  $\delta_{\text{H}}$  5.12 (1H, d,  $J$  0.8 Hz H-12b). The COSY spectrum showed the coupling of H-12a with the carbinolic hydrogens at  $\delta_{\text{H}}$  4.19 (1H, dd,  $J$  11.2; 0.8 Hz, H-13a) and  $\delta_{\text{H}}$  4.16 (1H, dd,  $J$  11.2; 0.8 Hz, H-13b). The peak attributed to H-8 at  $\delta_{\text{H}}$  3.88 (1H, ddd,  $J$  4.4; 10.0; 15.6 Hz) exhibited correlations in the COSY spectrum with the hydrogens at  $\delta_{\text{H}}$  2.43 (1H, ddd,  $J$  4.4; 8.0; 13.2 Hz, H-7),  $\delta_{\text{H}}$  2.18 (1H, dd,  $J$  10.0; 12.4 Hz, H-9a) and  $\delta_{\text{H}}$  1.38 (1H, m, H-9b).

The presence of a methine group at  $\delta_{\text{H}}$  1.71 (1H, m, H-4) was confirmed by COSY correlations at  $\delta_{\text{H}}$  1.21 (1H, m, H-3a) and  $\delta_{\text{H}}$  1.71 (1H, m, H-3b) and  $\delta_{\text{H}}$  0.71 (3H, d,  $J$  5.6 Hz,  $\text{CH}_3$ -15). The signal of  $\text{CH}_2$ -13 ( $\delta$  4.16 and 4.19),  $\text{CH}_3$ -14 ( $\delta$  1.02),  $\text{CH}_3$ -15 ( $\delta$  0.71) and  $\text{CH}_2$ -12 ( $\delta$  5.12 and 5.26) in the  $^1\text{H}$  NMR spectrum were in agreement with the profile of an eremophilane with a double bond at C-11 and C-12. The epoxide group at C-1 and C-10 was deduced from the chemical shifts of H-1 ( $\delta$  2.97), C-1 ( $\delta$  59.7), and



**Figure 2.** Selected H-H COSY and HMBC correlations for compound **2**.

C-10 ( $\delta$  65.8). The COSY analysis of **2** led to a partial structure as shown by bold-faced lines in Figure 2, which were supported by HMBC correlations (Table 2). The relative stereochemistry was based on those determined to compound **2**. All NMR data can be observed in Table 2.

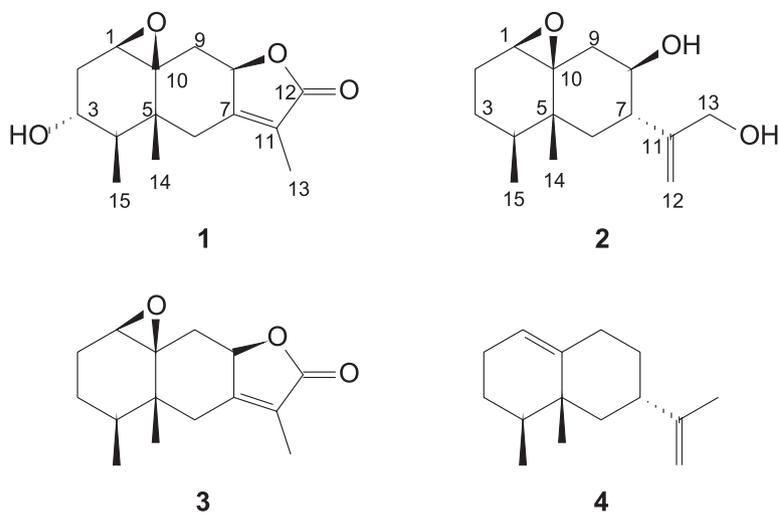
## Conclusions

The genus *Xylaria* is known for being a rich source of structurally diverse natural products including isocoumarin,<sup>3,4</sup> cytochalsins,<sup>4-6</sup> xyloketal,<sup>8-10</sup> sesquiterpenes<sup>11</sup> and others. Among these compounds eremophilane sesquiterpenes stand out for several biological activities.<sup>12-15</sup> One member of this genus reported in the present study showed the notable ability to produce eremophilane sesquiterpenes, including two new in the literature, cupressolide A and cupressolide B. Due to the many biological activities shown

**Table 2.** NMR spectroscopy data for eremophilane sesquiterpene **2**<sup>a</sup>

Position	$\delta_{\text{H}}$ , mult, ( $J$ in Hz)	$\delta_{\text{C}}$	COSY	HMBC(H $\rightarrow$ C)
1	2.97, d (6.8)	59.7	H2a/b	n.d.
2a/2b	1.91, m	22.1	H1, H3a/b	n.d.
3a/3b	1.21, m	24.3	H2a/b, H4	C15
4	1.71, m	33.1	H3a/b, H15	n.d.
5	-	35.8	-	C4, C15
6a	1.68, m	39.6	H6b, H7	C7, C8
6b	1.41, m		H6a, H7, H8	C7, C8, C10
7	2.43, ddd, (4.4; 8.0; 13.2)	45.4	H6a, H6b, H8,	n.d.
8	3.88, ddd (4.4; 10.0; 15.6)	71.6	H6b, H7, H9a/b	n.d.
9a	2.18, dd (10.0; 12.4)	38.5	H8, H9b	C8
9b	1.38 (m, 1H)		H9a	C7, C8, C10
10	-	65.8	-	-
11	-	150.1	-	-
12a	5.26, d (0.8)	113.2	12b, H13a, H13b	C7, C13
12b	5.12, d (0.8)		H12a	C7, C13
13a	4.19, dd (11.2; 0.8)	65.8	H12a	C7, C11, C12
13b	4.16, dd (11.2; 0.8)		H12a	C7, C11, C12
14	1.02, s	15.9	n.d.	C4, C5, C6, 10
15	0.71, d (5.6)	14.8	H4	C3, C4, C5

<sup>a</sup>The data were acquired at 400 and 100 MHz for  $^1\text{H}$  and  $^{13}\text{C}$  respectively in  $\text{CDCl}_3$ . TMS was used as internal reference.



**Figure 3.** Eremophilane sesquiterpenes produced by *Xylaria* sp., an endophytic fungus isolated from *Cupressus lusitanica* leaves.

by this class of compounds, this *Xylaria* deserves a careful study aiming to sesquiterpene production enhancement.

## Experimental

### Equipment

IR spectra were run on a Bomem MB102 -IR spectrometer using KBr pellets. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. GC/MS analyses were performed using GC 8000 series Fisons and VG Platform mass spectrometer detector. 1D and 2D NMR spectra were obtained in  $\text{CDCl}_3$  (Aldrich) on DRX 400 Bruker spectrometer operating at 400 MHz for hydrogen and 100 MHz for  $^{13}\text{C}$  and TMS was used as internal standard. MS were acquired in positive ion mode on a triple quadrupole Micromass Quattro LC spectrometer, equipped with an ESI ion source.

### Plant material

Health leaves of *Cupressus lusitanica* were collected in São Carlos, São Paulo State, Brazil. A voucher specimen (No. 7281) has been deposited in the Herbarium of the Botanic Department of Universidade Federal de São Carlos, Brazil.

### Fungal material

The method of surface sterilization employed in this work was similar to that used by Petrini *et al.*<sup>18</sup> After the collection, the leaves were washed in abundant water (domestic use grade) and then in distilled water. The leaves were surface sterilization by consecutive immersion in 70% ethanol (2 s), sterile distilled water (2 s), 11% aqueous sodium hypochlorite for 1-5 min and 70% ethanol (2 s),

and then in sterile distilled water. The material was placed in Petri dishes containing PDA medium (potato, dextrose and agar) supplemented with  $100 \mu\text{g mL}^{-1}$  terramycin and incubated at room temperature. Endophytic fungi growing from the plant tissues, were picked and recultured on PDA to determine culture purity. It was deposited at LaBioMMi – Laboratório de Bioquímica Micromolecular de Microorganismos – of the Departamento de Química at Universidade Federal de São Carlos, Brazil. Working stocks were prepared on potato dextrose agar.

### Fermentation and extraction

The fungus was grown under static conditions at room temperature for 20 days in 20 Erlenmeyer flasks containing the liquid medium (300 mL *per* flask) composed of glucose ( $26.7 \text{ g L}^{-1}$ ), yeast extract ( $10.0 \text{ g L}^{-1}$ ),  $\text{NaNO}_3$  ( $3.0 \text{ g L}^{-1}$ ),  $\text{K}_2\text{HPO}_4$  ( $1.0 \text{ g L}^{-1}$ ),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( $0.5 \text{ g L}^{-1}$ ),  $\text{KCl}$  ( $0.5 \text{ g L}^{-1}$ ),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ( $0.01 \text{ g L}^{-1}$ ). The mycelium was separated by reduced pressure filtration and the liquid phase was partitioned with ethyl acetate ( $1,000 \text{ mL} \times 3$ ). The organic solvent was dried with anhydrous sodium sulfate, filtered and removed using vacuum to give the crude extract. Crude extract was analyzed by GC/MS. This technique enabled the detection of the eremophilane sesquiterpene valencene (**4**). The extract was chromatographed on silica gel column ( $h = 4.5 \text{ cm}$  and  $\varnothing = 3.7 \text{ cm}$ ) eluted with a Hex: $\text{CH}_2\text{Cl}_2$  (1:1);  $\text{CH}_2\text{Cl}_2$ :EtOAc (1:1);  $\text{CH}_2\text{Cl}_2$ :EtOAc (1:4);  $\text{CH}_2\text{Cl}_2$ :EtOAc:MeOH (1:4:10%); EtOAc:MeOH (1:1); MeOH (100%) to afford six fractions (A-F). The fractions C and D were rechromatographed on silica gel column ( $h = 16 \text{ cm}$  and  $\varnothing = 2.5 \text{ cm}$ ) with Hexane 100% gradient to EtOAc:MeOH (1:1) to give **1** (5.2 mg), **2** (4.8 mg), **3** (2.7 mg).

### GC/MS analysis

The extract was submitted to clean-up procedures using solid phase extraction (SPE). The SPE cartridge was activated with 100% Hexane and conditioned with 3 mL of  $\text{CHCl}_3$ . The extract (10 mg) was dissolved in 3 mL of  $\text{CHCl}_3$  and loaded to the SPE cartridge. Elution of SPE cartridge with  $\text{CHCl}_3$  produced an apolar fraction. For the GC/MS analysis the injector temperature was kept at 180 °C and the GC oven temperature was maintained at 70 °C during 6 min and then increased to 250 °C at a rate of 6 °C  $\text{min}^{-1}$  and finally increased to 325 °C at 3 °C  $\text{min}^{-1}$ . The sample volume injected was 2  $\mu\text{L}$ .

### MS data collection

ESI-MS data were collected from direct introduction of the sample solution 5  $\mu\text{L}$  of compound **1** (5  $\mu\text{g mL}^{-1}$ ). The optimal voltages found for the probe and ion source components to produce maximum intensity of the ions  $[\text{M}+\text{H}]^+$  were 3.3 kV for the capillary, 19 V for the sample cone, and 4 V for the extractor cone.

### Physical and spectral data

(1 $\alpha$ R,3R,4R,4 $\alpha$ R,8 $\alpha$ R,9 $\alpha$ S)-3-Hydroxy-4,4 $\alpha$ ,6-trimethyl-1 $\alpha$ ,2,3,4,4 $\alpha$ R,8 $\alpha$ ,9-octahydro-7H-oxirene[8,8 $\alpha$ ]naphtho[2,3 $\beta$ ]furan-7-one, compound **1**: Cupressolide A

Colorless crystals ( $\text{CH}_2\text{Cl}_2$ );  $[\alpha]_{\text{D}}^{25} = -6.262$  (c 0.0001,  $\text{CHCl}_3$ ); IR(KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3487, 1745; ESI-MS  $m/z$  265  $[\text{M}+\text{H}]^+$ ; ESI-MS-MS (20 eV)  $m/z$  265 (78%), 247 (17), 229 (39), 201 (50), 183 (100), 173 (70), 147 (60), 119 (55); NMR data see Table 1.

(1 $\alpha$ R,4S,4 $\alpha$ R,6S,7R,8 $\alpha$ S)-6-[1-(Hydroxymethyl)vinyl]-4,4 $\alpha$ -dimethyloctahydro-1 $\alpha$ H-naphtho[1,8 $\alpha\beta$ ]oxiren-7-ol, compound **2**: Cupressolide B

Colorless crystal ( $\text{CH}_2\text{Cl}_2$ ); IR(KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$ : 3508; EIMS (70 eV)  $m/z$  252  $[\text{M}]^+$  (3%), 234 (6), 216 (12), 201 (16), 168 (57), 153 (75), 125 (100); NMR data see Table 2.

## Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbq.org.br>, as PDF file.

## Acknowledgments

The authors are grateful to the Brazilian institutions FAPESP – Fundação de Amparo à Pesquisa do Estado de

São Paulo, CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico, CAPES – Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior for the financial support.

## References

- Farjon, A.; *Taxon* **1993**, *42*, 81.
- Graniti, A.; *Annu. Rev. Phytopathol.* **1998**, *36*, 91.
- Tansuwan, S.; Pornpakakul, S.; Roengsumran, S.; Petsom, A.; Muangsin, N.; Sihanonta, P.; Chaichit N.; *J. Nat. Prod.* **2007**, *10*, 1620.
- Pongcharoen, W.; Rukachaisrikul, V.; Isaka, M.; Sriklung, K.; *Chem. Pharm. Bull.* **2007**, *55*, 1647.
- Espada, A.; Rivera-Sagredo, A.; de la Fuente, J. M.; Hueso-Rodríguez, J. A.; Élson, S. W.; *Tetrahedron* **1997**, *53*, 6485.
- Abate, D.; Abraham, W.; Meyer, H.; *Phytochemistry* **1997**, *44*, 1443.
- Healy, P. C.; Hocking, A.; Tran-Dinh, N.; Pitt, J. I.; Shivas, R. G.; Mitchell, J. K.; Kotiw, M.; Davis, R. A.; *Phytochemistry* **2004**, *65*, 2373.
- Lin, Y.; Wu, X.; Feng, S.; Jiang, G.; Luo, J.; Zhou, S.; Vrijmoed, L. L. P.; Jones, E. B. G.; Krohn, K.; Steingröver, K.; Zsila, F.; *J. Org. Chem.* **2001**, *66*, 6252.
- Xiaobo, Z.; Haiying, W.; Linyu, H.; Yongcheng, L.; Zhongtao, L.; *Process Biochem.* **2006**, *41*, 293.
- Liu, X.; Xu, F.; Zhang, Y.; Liu, L.; Huang, H.; Cai, X.; Lin, Y.; Chanb W.; *Russ. Chem. Bull.* **2006**, *55*, 1091.
- McDonald, L. A.; Barbieri, L. R.; Bernan, V. S.; Janso, J.; Lassota, P.; Carter G. T.; *J. Nat. Prod.* **2004**, *67*, 1565.
- Wang, W.; Gao, K.; Jia, Z.; *J. Nat. Prod.* **2002**, *65*, 714.
- Garduño-Ramírez, M. L.; Trejo, A.; Navarro, V.; Bye, R.; Linares, E.; Delgado, G.; *J. Nat. Prod.* **2001**, *64*, 432.
- Li, E.-W.; Pan, J.; Gao, K.; Jia, Z.-J.; *Planta Med.* **2005**, *71*, 1140.
- Zhang, Q. J.; Dou, H.; Zheng, Q. X.; Zhou, C. H.; Xu, Z. J.; Peng, H.; Zhao, Y.; *Chin. Chem. Lett.* **2005**, *16*, 360.
- Zhao Y.; Schenk, D. J.; Takahashi, S.; Chappell, J.; Coates, R. M.; *J. Org. Chem.* **2004**, *69*, 7428.
- Prez-Castorena, A. L.; Arciniegas, A.; Guzmán, S. L.; Villaseor, J. L.; de Vivar, A. R.; *J. Nat. Prod.* **2006**, *69*, 1471.
- Petrini, O.; Sieber, T. N.; Toti, L.; Viret, O.; *Natural Toxins* **1992**, *1*, 185

Received: June 25, 2009

Web Release Date: March 25, 2010

FAPESP has sponsored the publication of this article.

## Two Novel Eremophilane Sesquiterpenes from an Endophytic Xylariaceous Fungus Isolated from Leaves of *Cupressus lusitanica*

Luciana S. Amaral and Edson Rodrigues-Filho\*

Departamento de Química, Universidade Federal de São Carlos, CP 676,  
13565-905 São Carlos-SP, Brazil

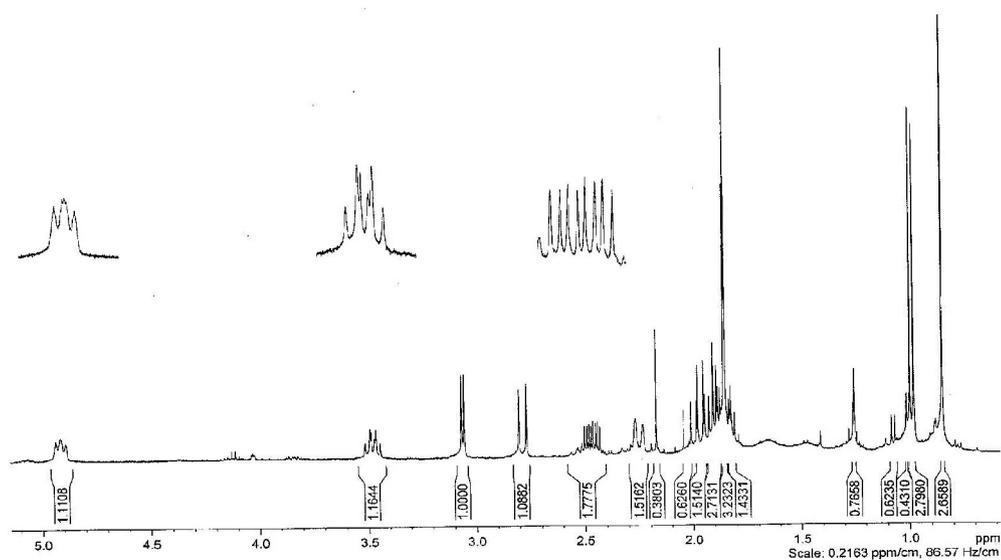


Figure S1. <sup>1</sup>H NMR spectrum of compound 1 (400 MHz, CDCl<sub>3</sub>).

\*e-mail: edson@dq.ufscar.br

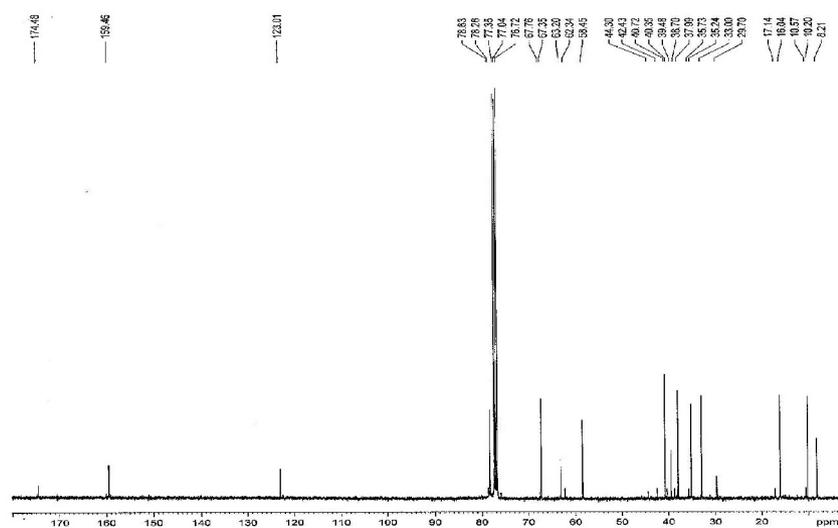


Figure S2. <sup>13</sup>C NMR spectrum of compound 1 (100 MHz, CDCl<sub>3</sub>).

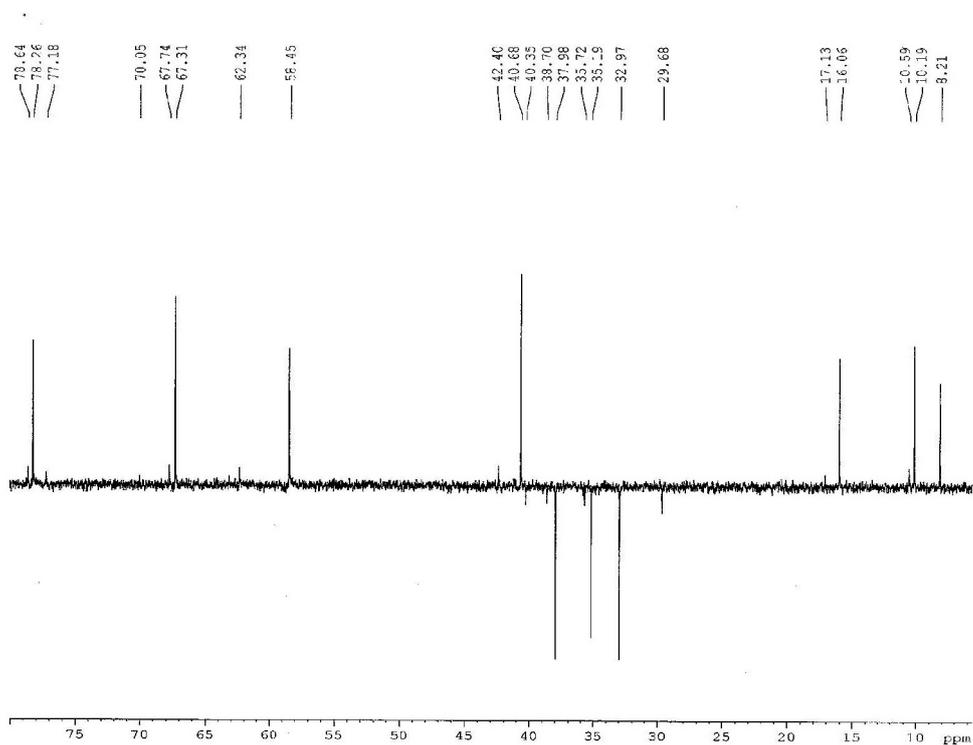
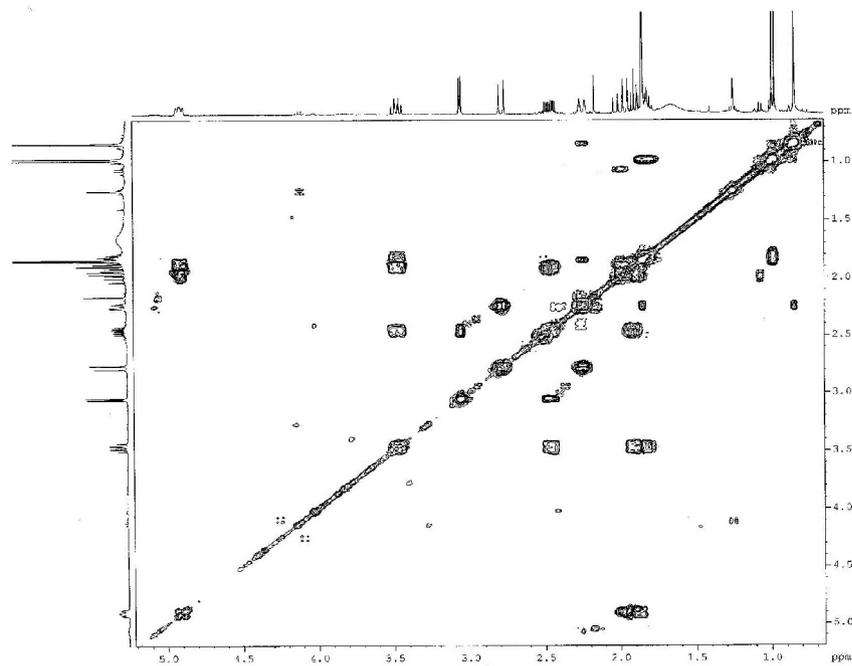
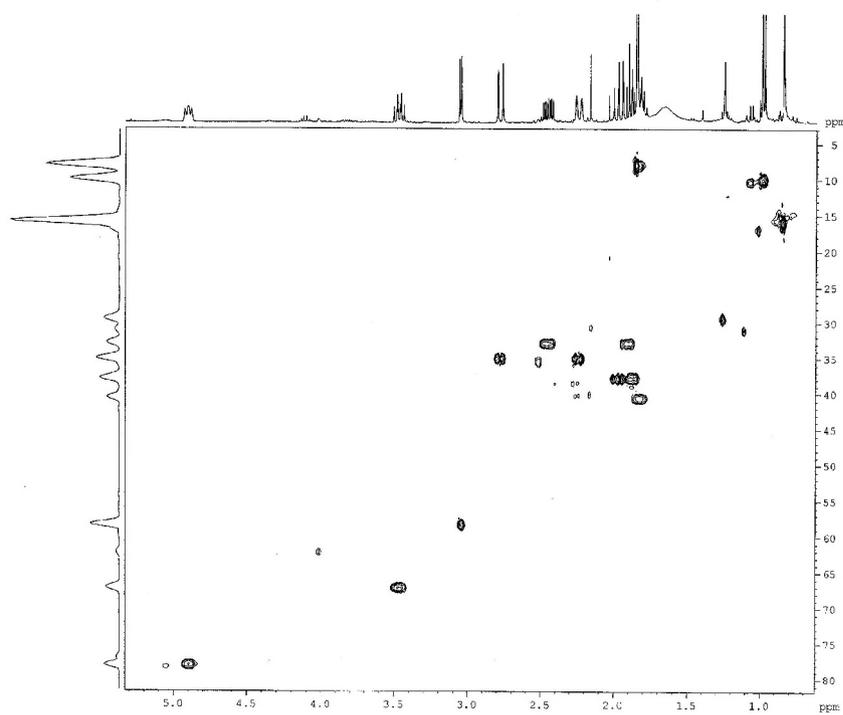


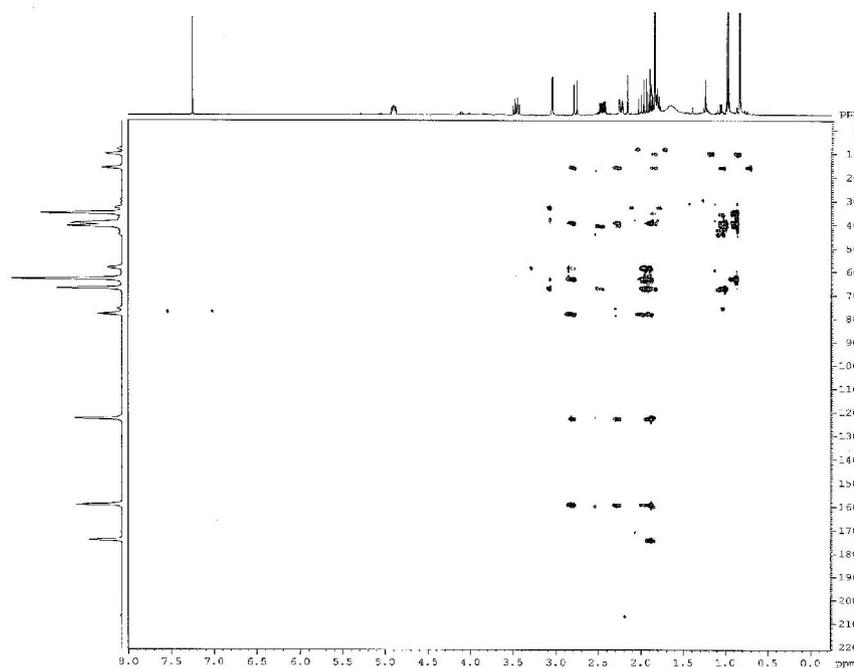
Figure S3. DEPT 135 spectrum of compound 1 (100 MHz, CDCl<sub>3</sub>).



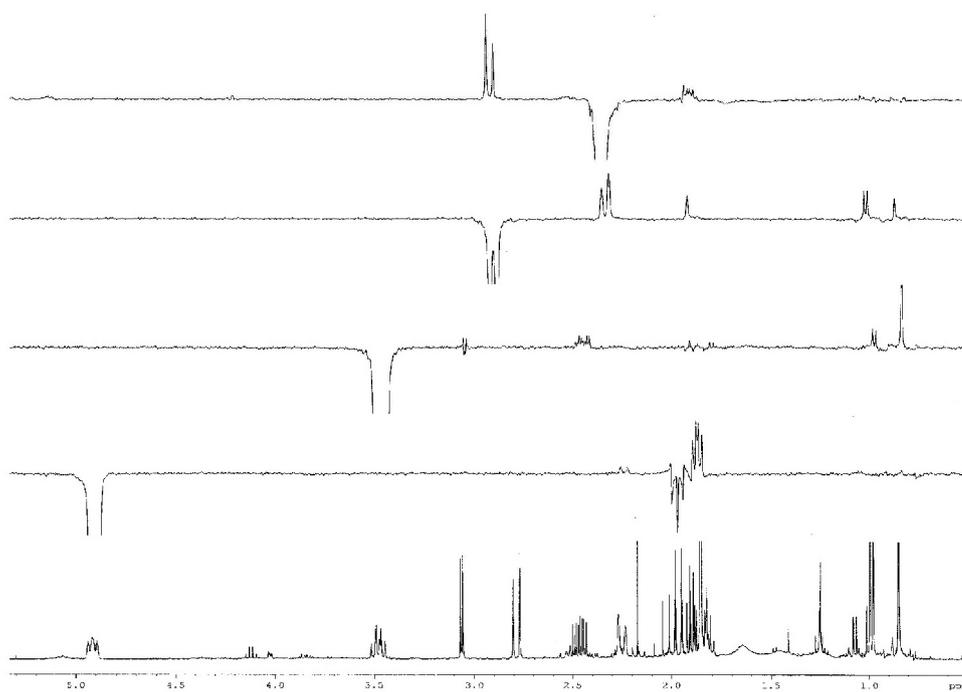
**Figure S4.** <sup>1</sup>H-<sup>1</sup>H COSY NMR correlation spectroscopy 2D NMR spectrum of compound **1** (400 MHz, CDCl<sub>3</sub>).



**Figure S5.** <sup>1</sup>H-<sup>13</sup>C HSQC 2D NMR correlation spectroscopy of compound **1** (400 MHz/100 MHz, CDCl<sub>3</sub>).



**Figure S6.**  $^1\text{H}$ - $^{13}\text{C}$  HMBC 2D NMR correlation spectroscopy of compound **1** (400 MHz/100 MHz,  $\text{CDCl}_3$ ).



**Figure S7.** nOe spectra for H-8, H-3, H-6b and H-6a of compound **1** (400 MHz,  $\text{CDCl}_3$ ).

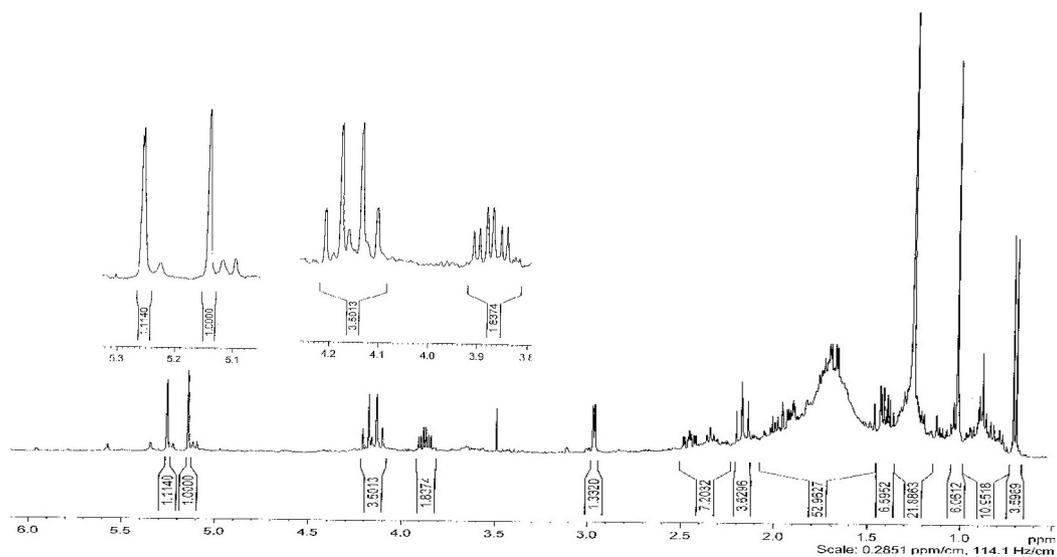


Figure S8.  $^1\text{H}$  NMR spectrum of compound **2** (400 MHz,  $\text{CDCl}_3$ ).

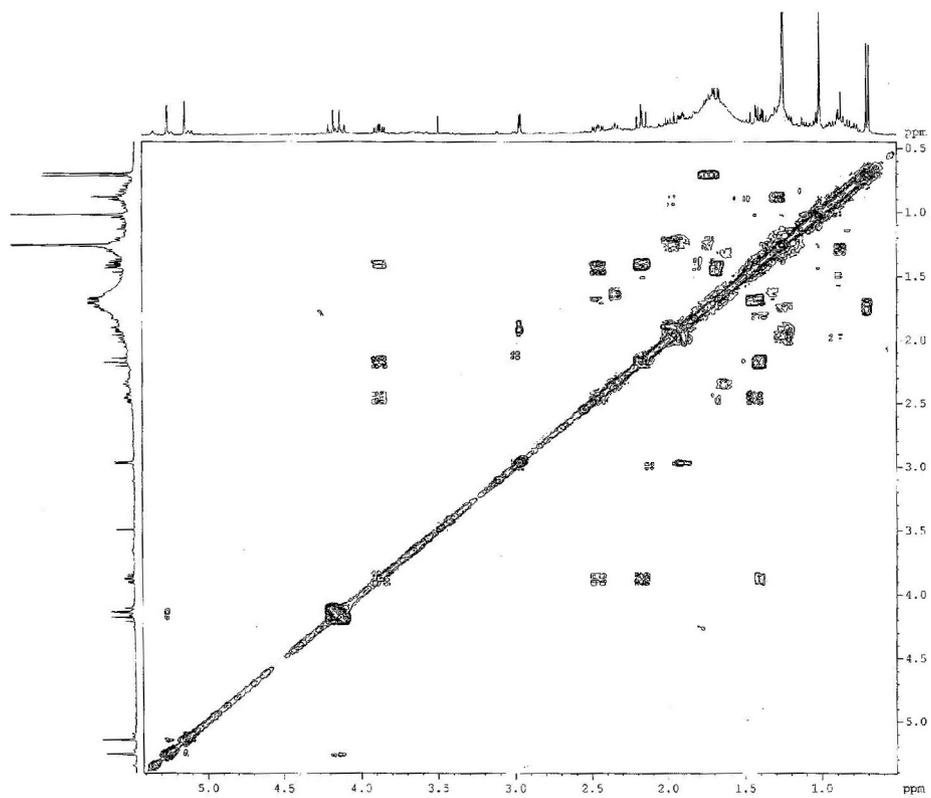


Figure S9.  $^1\text{H}$ - $^1\text{H}$  COSY NMR correlation spectroscopy 2D NMR spectrum of compound **2** (400 MHz,  $\text{CDCl}_3$ ).

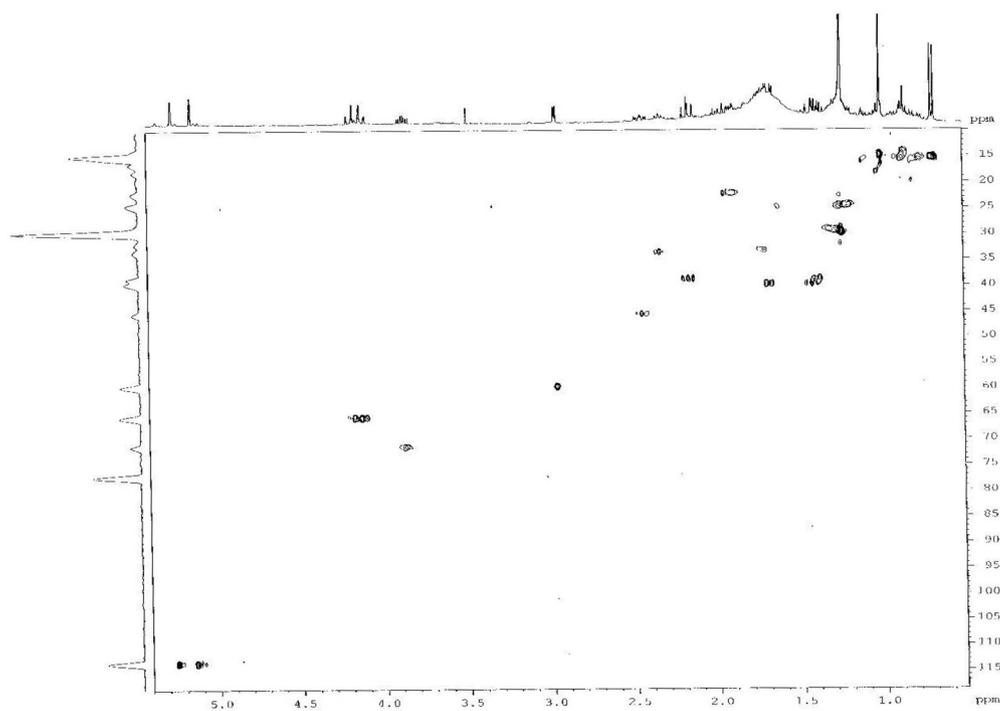


Figure S10.  $^1\text{H}$ - $^{13}\text{C}$  HSQC 2D NMR correlation spectroscopy of compound **2** (400 MHz/100 MHz,  $\text{CDCl}_3$ ).

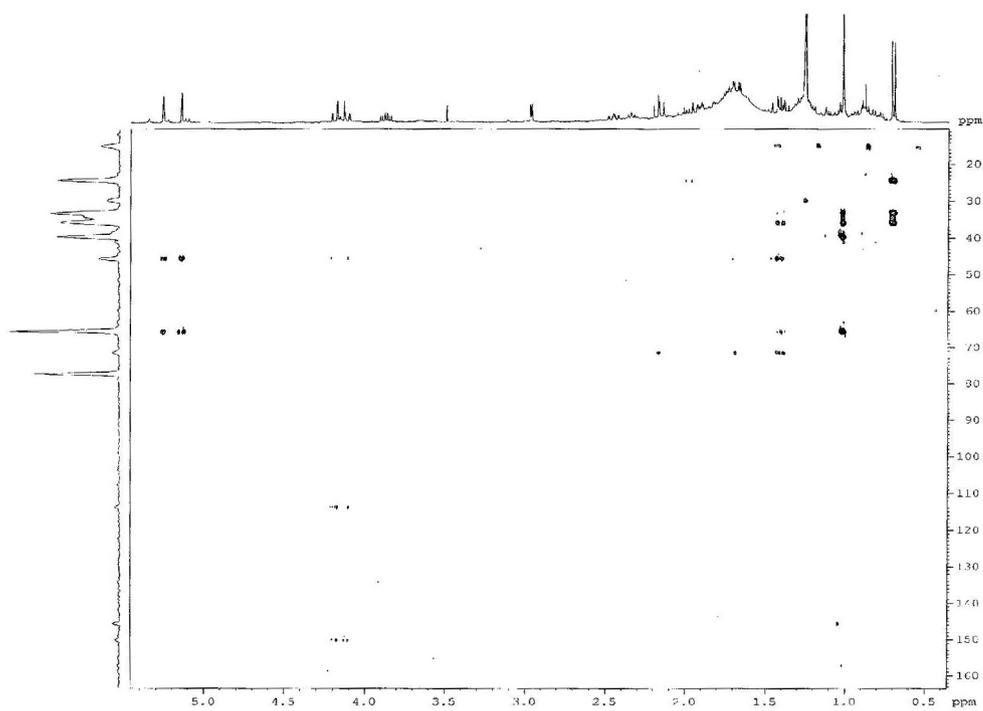


Figure S11.  $^1\text{H}$ - $^{13}\text{C}$  HMBC 2D NMR correlation spectroscopy of compound **2** (400 MHz/100 MHz,  $\text{CDCl}_3$ ).