

## Limonoids from Andiroba Oil and *Cedrela fissilis* and their Insecticidal Activity

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Nove limonóides foram isolados de *Carapa guianensis* e *Cedrela fissilis*. Entre eles, a 1,2-diidro-3 $\beta$ -hidroxi-7-desacetoxi-7-oxogedunina é uma substância inédita. Além disso, alguns deslocamentos químicos da xilocensina k foram corrigidos e os dados de RMN <sup>1</sup>H da 7-desacetilgedunina são descritos pela primeira vez na literatura. Seis dos limonóides isolados foram submetidos a ensaios com formigas *Atta sexdens rubropilosa* e apresentaram atividade inseticida moderada.

Nine limonoids were isolated from *Carapa guianensis* and *Cedrela fissilis*. Among them, 1,2-dihydro-3 $\beta$ -hydroxy-7-deacetoxy-7-oxogedunin is a new compound. Moreover, the assignments of some chemical shifts of xylocensin k have been corrected and <sup>1</sup>H NMR data of 7-deacetylgedunin have been assigned for the first time. These isolated limonoids were assayed on *Atta sexdens rubropilosa* workers showing moderate insecticidal activities.

**Keywords:** Meliaceae, *Carapa guianensis*, *Cedrela fissilis*, limonoids, insecticidal activity

### Introduction

The family Meliaceae includes many plants that are sources of valuable timber and many that have wide-ranging uses in ethnomedicine. The family is distinguished by the occurrence of characteristic substances called limonoids.<sup>1</sup> These substances have wide spectrum of biological activities,<sup>2</sup> particularly insecticidal action.<sup>3</sup>

The leaf-cutting ants of the genera *Atta* and *Acromyrmex* use mostly fresh plant fragments to raise their symbiotic fungi<sup>4,5</sup> and are the cause of considerable economic damage, due to defoliation that they cause.<sup>6</sup> Control of this pest is still problematic, presenting only temporary effects and is sometimes, harmful to the environment, to man and other animals.<sup>6,7</sup> Consequently, an extensive search for alternate methods to control these insects has been made in an attempt to substitute traditional agrochemicals with agents that yield faster decay, higher specificity and, therefore, less damaging to the environment. In this context, plants are considered a promising source for less toxic compounds that could be used as a soft control method.

As a part of our search for new natural insecticides against leaf-cutting ants<sup>8</sup> and because of the fact that limonoids have displayed biological activity towards a variety of insects, we decided to investigate the effect of limonoids isolated from two species of Meliaceae family, *Carapa guianensis* Aubl. and *Cedrela fissilis* Vell. on *Atta sexdens rubropilosa*.

*Carapa guianensis* is a tall tree that grows wild throughout South America, West India and South Africa. In Brazil, it can be found prevalently in areas of the Amazon rainforest, that are rich in soils and swamps. From the nuts of this plant is extracted an oil, called andiroba oil, which has a long history of traditional use in South America, such as analgesic, anti-inflammatory, insecticide, antibacterial, anti-parasitic and as an anti-cancer remedy.<sup>9-12</sup>

Andiroba oil is rich in fatty acids such as oleic, palmitic, stearic and linoleic acids, together with 2-5% of unsaponifiable material. In addition, from several parts of *Carapa guianensis* have been isolated limonoids, triterpenes, steroids, coumarins, flavonoids and diglycerides.<sup>13-18</sup>

*C. fissilis* tree, known as “cedro” (cedar), is a valuable source of timber. In Brazil, it can be found from the Amazon forest as far south as Espírito Santo State. Previous investigation of fruits and seeds from this species

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afforded the limonoids fassinolide, mexicanolide and 3 $\beta$ -hydroxyisomexicanolide.<sup>19</sup>

Extracts and fractions from *C. fissilis* and one commercial *C. guianensis* oil showed to have activity against *A. sexdens rubropilosa* workers.<sup>20,21</sup> In the herein study we report the insecticidal activities towards *A. sexdens rubropilosa* of six gedunin derivatives limonoids obtained from these active fractions besides spectroscopic data for 1,2-dihydro-3 $\beta$ -hydroxy-7-deacetoxy-7-oxogedunin (**5**), 7-deacetylgedunin (**6**) and xylocensin k (**9**).

## Results and Discussion

The chemical investigation of one commercial andiroba oil sample afforded seven limonoids: 17 $\beta$ -hydroxyazadiradione (**1**), gedunin (**2**), 6 $\alpha$ -acetoxygedunin (**3**), 7-deacetoxy-7-oxogedunin (**4**), 1,2-dihydro-3 $\beta$ -hydroxy-7-deacetoxy-7-oxogedunin (**5**), methyl angolensate (**8**) and xylocensin k (**9**). Moreover, from roots and leaves of *C. fissilis* were isolated 7-deacetylgedunin (**6**) and photogedunin (**7**) along with limonoids **2**, **3**, **4** and **5**.

Compounds **1**, **2** and **9** have been isolated for the first time from *C. guianensis* and all the limonoids isolated from *C. fissilis* are new for this species. Compound **5** is new to the literature.

Limonoids **1**,<sup>22</sup> **2**,<sup>23</sup> **3**,<sup>13</sup> **4**,<sup>15</sup> **7**,<sup>24</sup> and **8**<sup>25</sup> were identified by comparison of their NMR spectral data to those previously reported in the literature. The <sup>1</sup>H NMR data of compound **6** (Table 1) supplement and expand some hydrogen and carbon chemical shifts reported by Ekong and Olagbemi.<sup>26</sup>

Compound **5** was isolated as a white powder from *C. fissilis* leaves and from andiroba oil and identified by spectroscopic methods. It is a new compound, whose 3-OH epimer was obtained from *Guarea thompsonii*.<sup>27</sup>

The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **5** exhibited signals at:  $\delta_{\text{H}}$  7.40 (m), 7.36 (m), 6.36 (s) and  $\delta_{\text{C}}$  143.0, 141.0, 120.4, 109.9 of a  $\beta$ -furan ring;  $\delta_{\text{H}}$  1.13 (s), 1.12 (s), 1.08 (s), 0.98 (s), 0.84 (s) and  $\delta_{\text{C}}$  27.4, 20.9, 17.1, 16.9, 14.8 related to five *tert*-methyl groups;  $\delta_{\text{H}}$  5.45 (s), 3.83 (s) and  $\delta_{\text{C}}$  167.3, 65.7, 53.6, 78.1 corresponding to a lactone D-ring with an epoxide between C-14 and C-15. These features are characteristics of compounds classified in the gedunin group of limonoids. However, the NMR spectra of compound **5** lacked signals related to the A-ring  $\alpha,\beta$ -unsaturated ketone. In contrast, signals were observed at  $\delta_{\text{H}}$  3.26 (dd, 11.0 and 5.0 Hz) and  $\delta_{\text{C}}$  78.2. These signals indicated an equatorial hydroxyl group at C-3. Thus, limonoid **5** was identified as 1,2-dihydro-3 $\beta$ -hydroxy-7-deacetoxy-7-oxogedunin and its spectral data allocations

**Table 1.** NMR spectral data of limonoids **5** and **6**

H/C	$\delta_{\text{H}}$ of <b>5</b> <sup>a</sup>	$\delta_{\text{C}}$ of <b>5</b> <sup>b</sup>	$\delta_{\text{H}}$ of <b>6</b> <sup>b</sup>
1		38.3	7.11 d ( <i>J</i> 10.2 Hz)
2	1.75 m	26.9	5.85 d ( <i>J</i> 10.2 Hz)
3	3.26 dd ( <i>J</i> 11.0; 5.0 Hz)	78.2	
4		39.5	
5	1.29 dd ( <i>J</i> 14.1; 3.1 Hz)	56.8	2.49 dd ( <i>J</i> 13.4; 2.4 Hz)
6	6 $\alpha$ : 2.40 dd ( <i>J</i> 13.9; 3.1 Hz) 6 $\beta$ : 2.75 dd ( <i>J</i> 14.1; 13.9 Hz)	36.5	1.92 m, 1.83 m
7		210.0	3.58 br s
8		52.9	
9	1.90 m	52.4	2.58 m
10		37.5 or 37.6	
11	1.60 m	16.8	2.00 m, 1.81 m
12		32.4	1.70 m, 1.57 m
13		37.5 or 37.6	
14		65.7	
15	3.83 s	53.6	3.91 s
16		167.3	
17	5.45 s	78.1	5.60 s
18	1.12 s	20.9	1.24 s
19	1.08 s	16.9	1.20 s
20		120.4	
21	7.40 m	141.0	7.41 m
22	6.36 s	109.9	6.35 m
23	7.36 m	143.0	7.41 m
28	0.84 or 0.98 s	14.8 or 27.4	1.09 s
29	0.84 or 0.98 s	14.8 or 27.4	1.10 s
30	1.13 s	17.1	1.15 s

<sup>a</sup>Spectra acquired in CDCl<sub>3</sub> at 400 MHz; <sup>b</sup>Spectra acquired in CDCl<sub>3</sub> at 100 MHz; coupling constant *J* in parenthesis.

(Table 1) were established unequivocally through 1D and 2D NMR experiments.

Compound **9** was isolated as a white powder from andiroba oil. It is classified as belonging to the mexicanolide group of limonoids and contains a tetrahydrofuran sub-unit with an oxygen bridge linking from C-3 to C-8. It was also isolated from the seed of *Xylocarpus granatum*.<sup>28</sup> However, we found through 2D NMR spectra analysis that some chemical shifts were incorrectly determined by Kokpol *et al.*,<sup>28</sup> among them H-17, H-28, H-29, C-8, C-17, C-18 and C-29.

The chemical shift of C-17 was deduced from <sup>1</sup>J correlation between H-17 ( $\delta$  6.28, s) and one carbon at  $\delta$  76.5. H-18 ( $\delta$  1.00, s) was assigned through <sup>3</sup>J correlation between the methyl hydrogens and carbinolic carbon at  $\delta$  76.5 (C-17). Furthermore, H-28 ( $\delta$  1.12) and H-29 ( $\delta$  0.67) were determined through <sup>3</sup>J correlation to C-3 ( $\delta$  91.4). Finally, the <sup>2</sup>J-<sup>3</sup>J correlations among H-30 ( $\delta$  2.52, dd, 12.4 and 6.1 Hz), H-2 ( $\delta$  2.98, br t, 6.1 Hz) and a quaternary carbon at  $\delta$  85.2 established C-8.

Some of the limonoids isolated from *C. guianensis* and *C. fissilis* were evaluated for their effects on *A. sexdens rubropilosa* ants. The results (Table 3) showed that control and limonoid-treated groups had on average the same

Table 2. NMR spectral data of limonoid **9**

Carbon	HSQC		HMBC	
	$\delta_C$	$\delta_H$ ( $^1J_{C-H}$ )	$^2J_{C-H}$	$^3J_{C-H}$
1	214.9			
2	49.0	2.98 br t ( $J$ 6.1 Hz)	214.9	85.2
3	91.4	4.23 d ( $J$ 6.1 Hz)		43.0, 214.9
4	37.2			
5	43.0	3.08 dd ( $J$ 10.7; 2.4 Hz)		
6	32.6	2.23 d ( $J$ 10.7 Hz)		37.2
7	174.4			
8	85.2			
9	52.2	1.97 dd ( $J$ 12.4; 4.7 Hz)		
10	51.1			
11	17.9	1.46 m, 2.10 m		
12	28.8	1.50 m, 1.70 m		
13	40.0			
14	74.7			
15	37.3	2.52 d ( $J$ 17.8 Hz), 3.16 d ( $J$ 17.8 Hz)	74.7, 169.9	
16	169.9			
17	76.5	6.28 s	120.1	143.0
18	16.2	1.00 s	40.0	28.8, 74.7, 76.5
19	16.9	0.95 s	51.1	43.0, 52.2, 214.9
20	120.1			
21	143.0	7.45 br s		
22	110.0	6.49 br s		
23	140.9	7.56 br s		
28	28.1	1.12 s	37.2	20.2, 43.0, 91.4
29	20.2	0.67 s	37.2	28.1, 43.0, 91.4
30	42.5	2.04 d ( $J$ 12.4 Hz), 2.52 dd ( $J$ 12.4, 6.1 Hz)	49.0, 85.2	52.2, 214.9
COOCH <sub>3</sub>	52.0	3.70 s		174.4
COOCH <sub>3</sub>	174.4			

Spectra acquired in CDCl<sub>3</sub>, 9.8 T; coupling constant  $J$  in parenthesis.

survival medium time. Compound **3** showed significant difference to the control according to the log-rank test ( $p < 0.05$ ), which takes into account the whole test period. The gedunin type limonoids do not seem to be highly active against these ants. Indeed, *C-seco* limonoids, such as azadirachtin, are more active insecticidal compound, possessing both antifeedant and growth-regulating activities.<sup>2,29</sup> The toxicity for the ants observed for the *C. fissilis* and *C. guianensis* extracts seems not to be related only to the presence of the limonoids. Perhaps the limonoids act in synergism between them or with other compounds in the extracts.

## Experimental

### General experimental procedures

The <sup>1</sup>H NMR, <sup>13</sup>C NMR and 2D correlation spectra were obtained in CDCl<sub>3</sub> using Bruker DRX-200 and ARX-400 NMR spectrometers, and using tetramethylsilane (TMS) as internal standard. HPLC was carried out using a preparative LC-8A Shimadzu system with SPD-6AV Shimadzu UV detector.

### Plant material

The roots and leaves of *Cedrela fissilis* Vell. were collected in São Carlos-SP, Brazil in June 01, 2001 and identified by Dr. Maria Inês Salgueiro Lima from the Department of Botany, Universidade Federal de São Carlos, where a voucher specimen (6701) was deposited. The commercial andiroba oil sample was purchased in Belém city (Pará, Brazil), in 1996.

### Isolation of the limonoids from *Carapa guianensis*

A portion of andiroba oil sample (21.0 g) was submitted to vacuum liquid chromatography (VLC) on silica gel (70-230 mesh) using a hexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-MeOH gradient, to yield the four corresponding fractions (H, D, Ac, Me). The fraction Ac (2.6 g) was subjected to column chromatography over silica gel (230-400 mesh, 267 x 38 mm i.d., stepwise with a hexane→MeOH gradient) to give three fractions. The second fraction (Ac2; 2.3 g) was chromatographed on a silica gel column (230-400 mesh, 264 x 45 mm i.d.) and eluted with solvents of increasing polarity

**Table 3.** Activities of gedunin limonoids on *Atta sexdens rubropilosa* workers at 100  $\mu\text{g mL}^{-1}$ 

Limonoid	Survival median ( $S_{50}$ ) / days
2	8
3	8 <sup>a</sup>
4	11
5	9
6	9
7	9
control	10

<sup>a</sup>Significant difference according to the log-rank test ( $p < 0.05$ ).

(hexane $\rightarrow$ MeOH) yielding five fractions. Limonoid **5** (Ac2,4,2; 17.7 mg) was isolated from fraction four (Ac2,4; 458.2 mg) using column chromatography over silica gel (230-400 mesh, 444 x 24 mm i.d., stepwise with a hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH gradient). The fifth fraction (Ac2,4,5; 409.0 mg) of this last step was subjected to column chromatography over silica gel (230-400 mesh, 475 x 24 mm i.d., stepwise with a hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH gradient) to give seven fractions. Fraction three (Ac2,4,5,3; 19.8 mg) was subjected to preparative HPLC (Hypersil 5 $\mu$  column, 290 x 10 mm i.d., eluted with hexane:isopropanol (9:1), flow rate: 3 mL min<sup>-1</sup>, detector UV at 240 nm) and the limonoid **9** (Ac2,4,5,3,4; 2.7 mg) was isolated.

Another portion of the andiroba oil sample (150.0 g) was submitted to VLC on silica gel (70-230 mesh) using a hexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-MeOH gradient, to yield four fractions (FH, FD, FA, FM). Fraction FA (19.0 g) was subjected to column chromatography over silica gel (230-400 mesh, 309 x 38 mm i.d., stepwise with a hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH gradient) to give nine fractions. Several chromatographies of fractions five, seven and eight (FA5, FA7 and FA8) afforded limonoids **1**, **2**, **3**, **4**, **8**, as described below.

The fifth fraction (FA5; 1.1 g) was chromatographed on a silica gel column (230-400 mesh, 269 x 32 mm i.d.) and eluted with solvents of increasing polarity (hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH) to afford nine fractions. The limonoids **2** (FA5,6,4; 4.5 mg) and **3** (FA5,6,3; 1.5 mg) were isolated from fraction six (FA5,6; 30.1 mg) by preparative HPLC (Asahipak GS-310 2G polymeric column, 500 x 22 mm i.d., eluted with MeOH (100%), flow rate: 5 mL min<sup>-1</sup>, detector UV at 220 nm).

Fraction seven (FA7; 338.4 mg) was chromatographed on a silica gel column (230-400 mesh, 466 x 24 mm i.d.) and eluted with solvents of increasing polarity (hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH) to afford seven fractions. The fourth fraction (FA7,4; 164.0 mg) was subjected to column chromatography over silica gel (230-400 mesh, 420 x 24 mm i.d.) and eluted with solvents of increasing polarity (hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH) to yield five fractions.

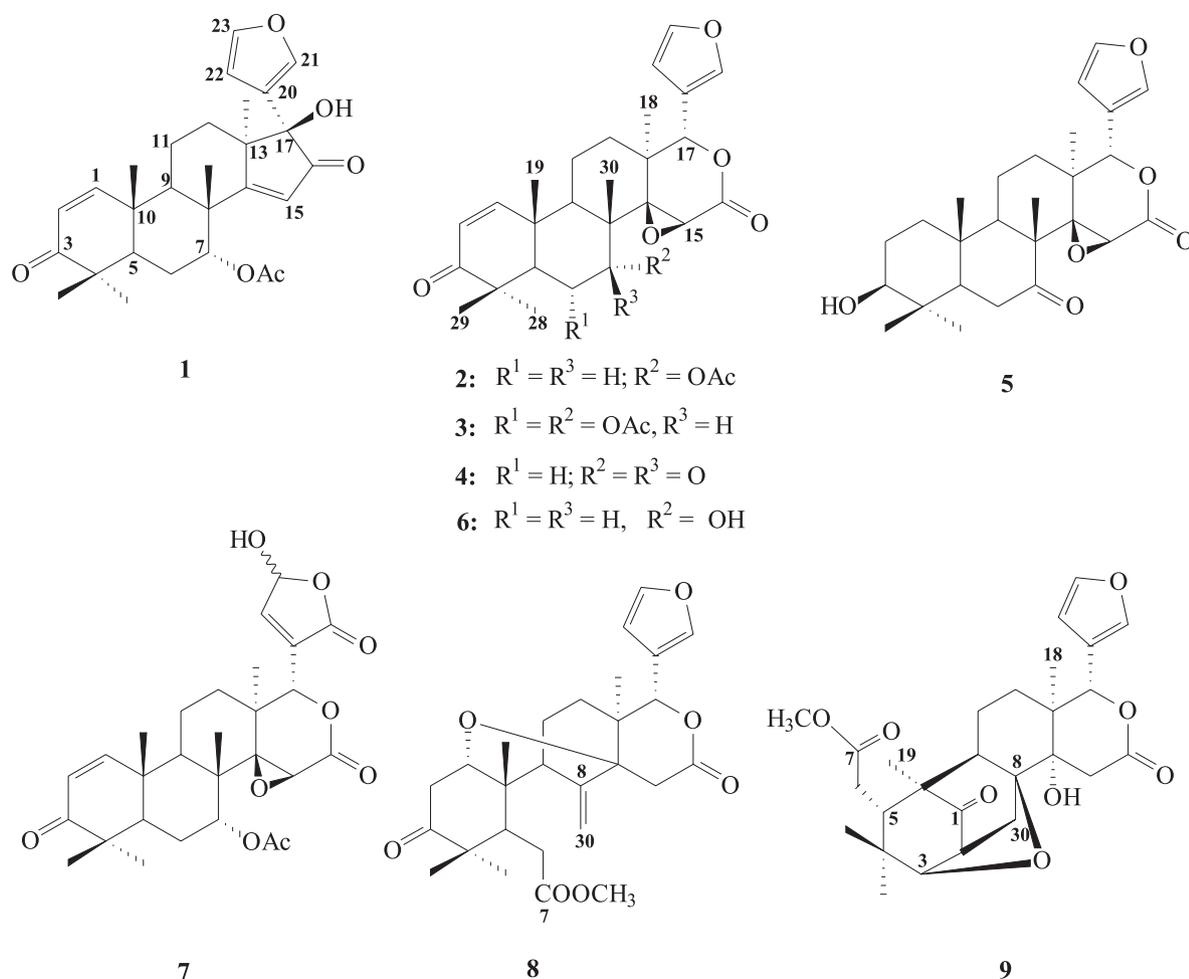
Through preparative HPLC (Asahipak GS-310 2G polymeric column, 500 x 22 mm i.d., eluted with MeOH (100%), flow rate: 5 mL min<sup>-1</sup>, detector UV at 220 nm) of fraction two (FA7,4,2; 38.9 mg) were isolated the limonoids **4** (FA7,4,2,3; 5.8 mg) and **8** (FA7,4,2,4; 17.4 mg).

Fraction eight (FA8; 342.9 mg) was chromatographed on a silica gel column (230-400 mesh, 342 x 24 mm i.d.) and eluted with solvents of increasing polarity (hexane:CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH) to afford six fractions. The fourth fraction (FA8,4; 195.4 mg) was subjected to column chromatography over silica gel (230-400 mesh, 473 x 24 mm i.d.) and eluted with solvents of increasing polarity (CH<sub>2</sub>Cl<sub>2</sub>:acetone $\rightarrow$ MeOH) to yield nine fractions. Limonoid **1** (FA8,4,6,2; 6.6 mg) was isolated from fraction six (FA8,4,6; 15.3 mg) through preparative HPLC (Hypersil 5m column, 290 x 10 mm i.d., eluted with hexane:isopropanol (9:1), flow rate: 3 mL min<sup>-1</sup>, detector UV at 240 nm).

#### Isolation of the limonoids from *Cedrela fissilis*

The powdered air-dried roots from *C. fissilis* were extracted by maceration for three times (72 h) with hexane, dichloromethane and and/or methanol at room temperature. The solvent was removed under reduced pressure by rotary evaporation. A portion of the hexane extract (3.5 g) from roots of *C. fissilis* (RH) was submitted to VLC on silica gel (70-230 mesh) using a hexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-MeOH gradient. The ethyl acetate-soluble fraction (704 mg) was chromatographed on silica gel (230-400 mesh), and eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub>-acetone (6:3:1) to give 5 fractions (A $\rightarrow$ E). Fraction E was twice chromatographed as above to give four fractions. Fraction E-4 was submitted to HPLC [Asahipak GS-310 2G polymeric column, 500 x 22 mm i.d., eluted with MeOH (100%), at a flow rate of 5 mL min<sup>-1</sup>, detector UV at 254 nm] affording **2** (13.1 mg) and **3** (2.5 mg).

The powdered air-dried leaves from *C. fissilis* were extracted by maceration as described above. The CH<sub>2</sub>Cl<sub>2</sub> extract (29.0 g) from leaves of *C. fissilis* (LH) was submitted to VLC as described to the hexane extract from roots (RH). The CH<sub>2</sub>Cl<sub>2</sub>-soluble fraction (6.0 g) was chromatographed on silica gel (70-230 mesh)-florisil (1:1), with a hexane-CH<sub>2</sub>Cl<sub>2</sub>-MeOH gradient to afford 9 fractions (A $\rightarrow$ I). Fraction D was three times chromatographed with hexane-CH<sub>2</sub>Cl<sub>2</sub>-MeOH (6:3.5:0.5) to give **4** (8.3 mg) and **6** (5.1 mg). Fraction I was also chromatographed three times over silica gel (230-400 mesh), eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub>-acetone (7:2:1) yielding 9 fractions. Fraction I-3 was purified by HPLC [Hypersil 5  $\mu$  column, 290 x 10 mm i.d., eluted with hexane:isopropanol (8:2), flow rate: 2.5 mL min<sup>-1</sup>,



**Figure 1.** Limonoids isolated from *Carapa guianensis* and *Cedrela fissilis*.

detector UV at 240 nm) to give **5** (1.2 mg). Fraction **I-8** was chromatographed on Sephadex LH-20, with MeOH (100%) to afford **7** (10.3 mg).

#### *1,2-dihydro-3 $\beta$ -hydroxy-7-deacetoxy-7-oxogedunin (5)*

White powder;  $[\alpha]_D^{25} = -72.8^\circ$  ( $c$  0.32,  $CH_2Cl_2$ ); UV ( $CH_2Cl_2$ )  $\lambda_{max}/nm$ : 236; IR (film)  $\nu_{max}/cm^{-1}$ : 3054, 2986, 1741, 1709, 1265, 1029;  $^1H$  NMR ( $CDCl_3$ , 400 MHz): Table 1;  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz): Table 1.

#### Bioassays

The *A. sexdens rubropilosa* workers used in the assays were randomly removed from laboratory nests. They had a combined body mass of 20-25 mg. Before assaying the nests were supplied daily with leaves of *Eucalyptus* sp., oat seeds and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp. or rose petals. Fifty ants were randomly removed from the nests and distributed to five Petri dishes (ten ants each) for each treatment. During the assays the ants

were maintained on an artificial diet consisting of glucose (50 g  $L^{-1}$ ), bacto-peptone (10 g  $L^{-1}$ ), yeast extract (1.0 g  $L^{-1}$ ) and agar (15 g  $L^{-1}$ ) in distilled water (100 mL).<sup>30</sup> The diets (0.4-0.5 g per dish) with the addition of limonoids (experiment) or without (control) were supplemented daily in small plastic caps. The control was prepared with the diet and the solvent. The compounds were poured into the hot diet immediately after it was autoclaved. The final concentration of the limonoids added to the diet was 100  $\mu g mL^{-1}$ . To ensure that undetectable remaining amounts of the solvent did not affect the ants, a comparison was made with another set of dishes in which water was used instead of solvent. As expected, the same survival rates were obtained with both systems (data not shown). During the assays the material was maintained in an incubator at a temperature of 25 ( $\pm 1$ )  $^\circ C$  and relative humidity ranging between 70-80%. The maximum length of observation was 25 days and the number of dead ants was recorded daily.

The survival average 50% ( $S_{50}$ ) was calculated and survival curves were compared by the computer-assisted software Graph-Pad™ using the log-rank test.

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## Supplementary Information

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

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## Limonoids from Andiroba Oil and *Cedrela fissilis* and their Insecticidal Activity

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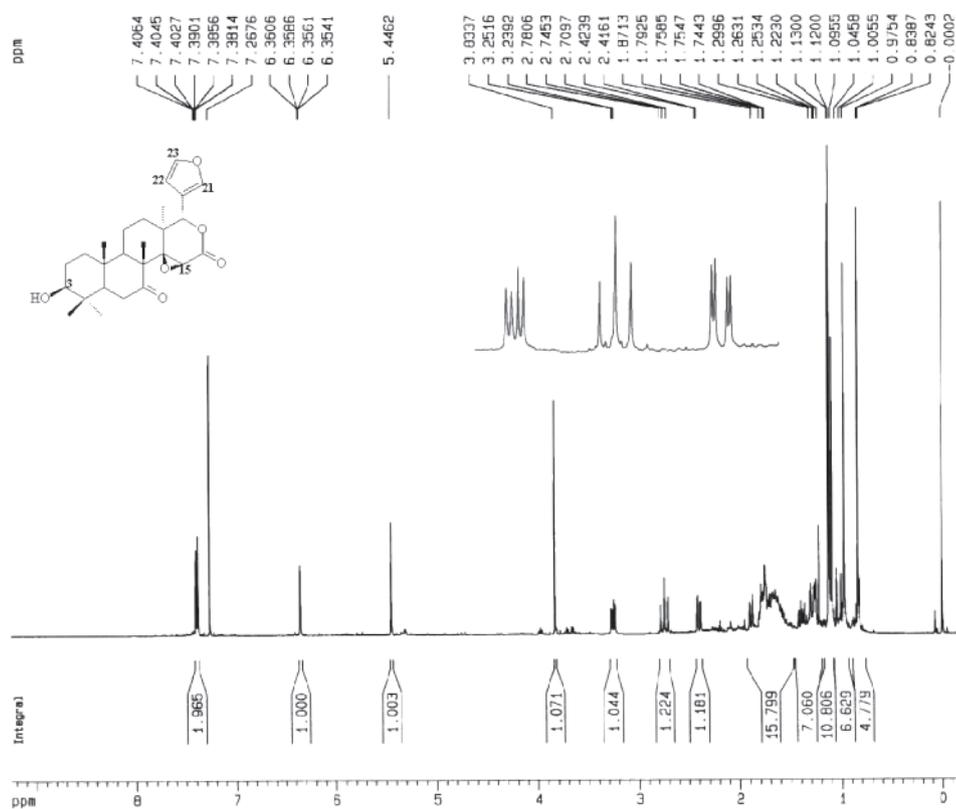


Figure S1. NMR <sup>1</sup>H spectra of 5 (400 MHz, CDCl<sub>3</sub>).

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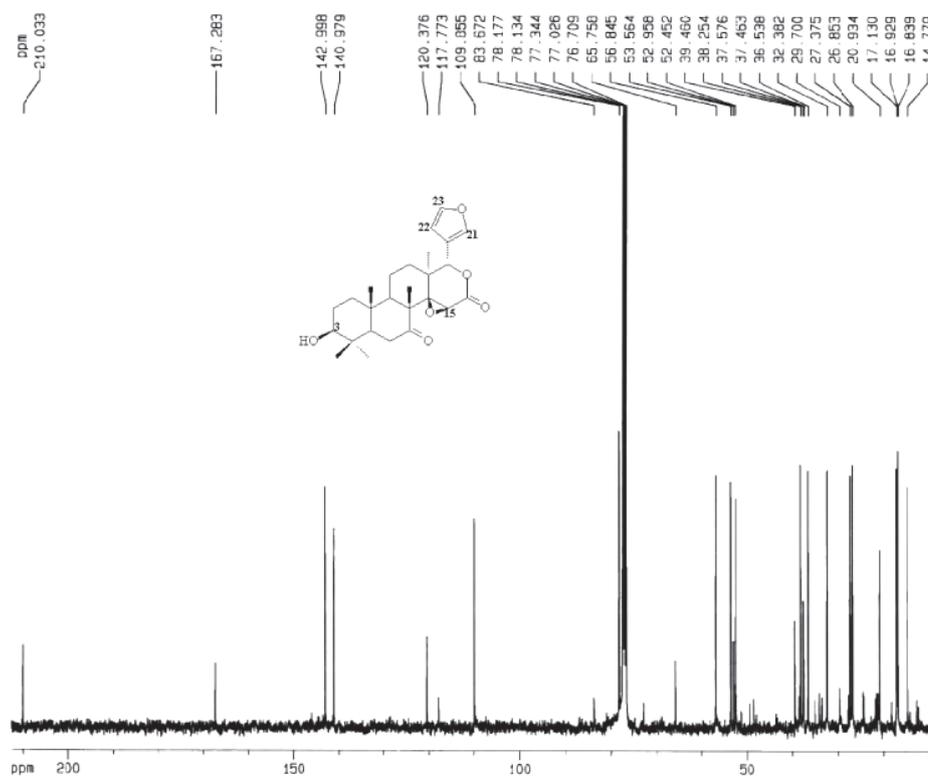


Figure S2. NMR  $^{13}\text{C}$  spectra of **5** (100 MHz,  $\text{CDCl}_3$ ).

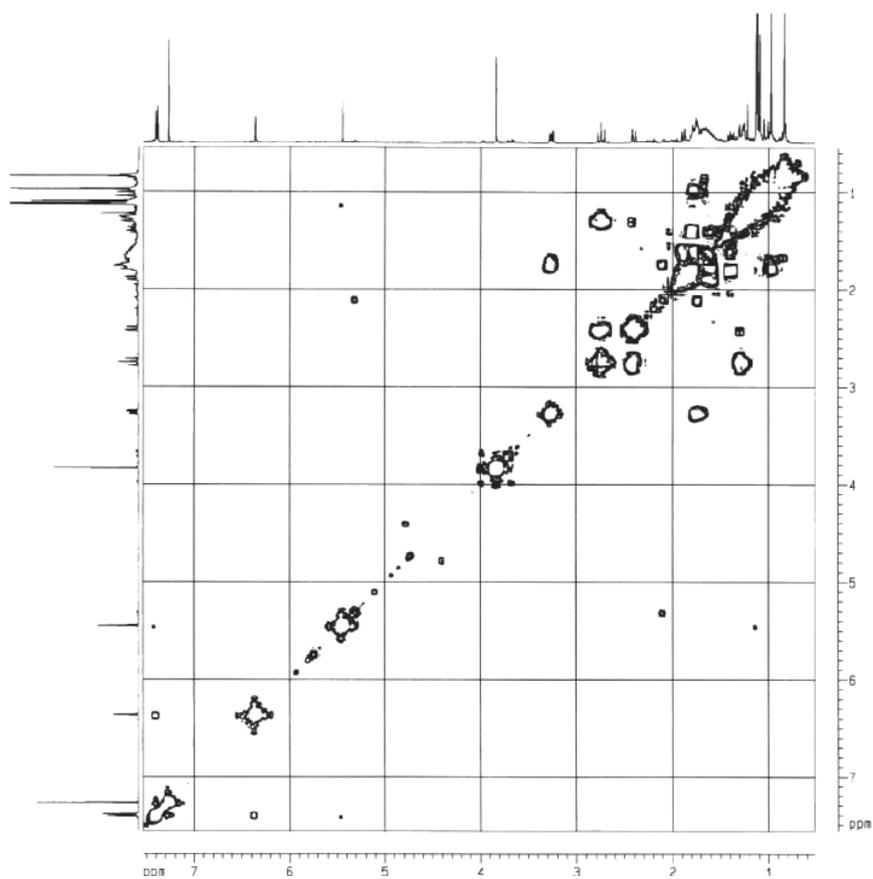


Figure S3. COSY  $^1\text{H}$ - $^1\text{H}$  45° spectra of **5** (400 MHz,  $\text{CDCl}_3$ ) A.

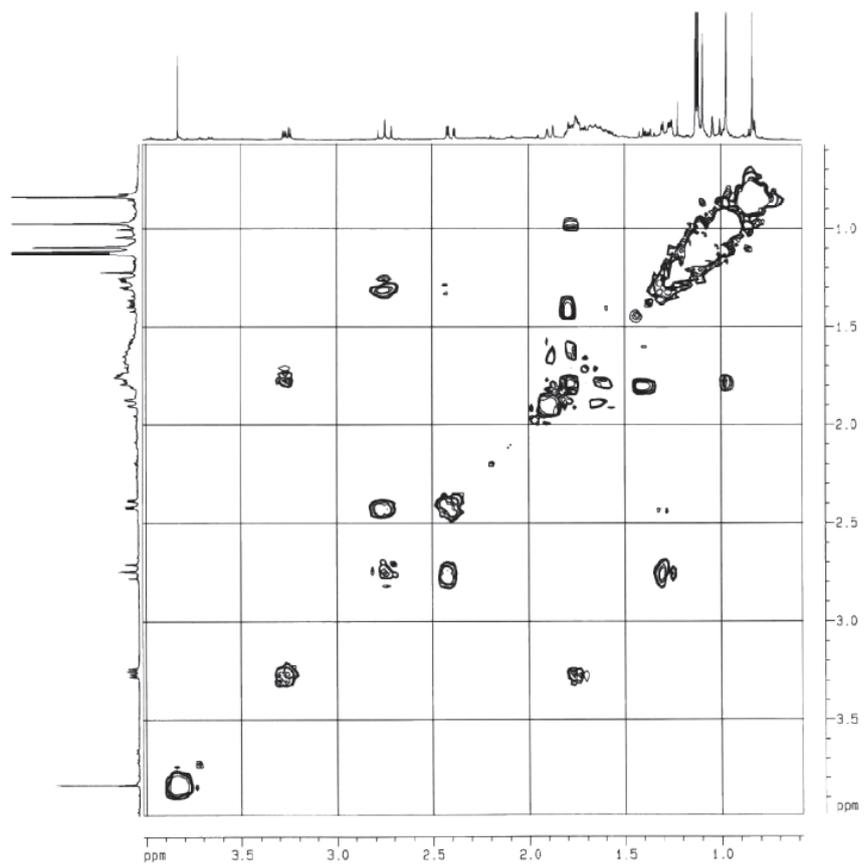


Figure S4. COSY <sup>1</sup>H-<sup>1</sup>H 45° spectra of **5** (400 MHz, CDCl<sub>3</sub>) B.

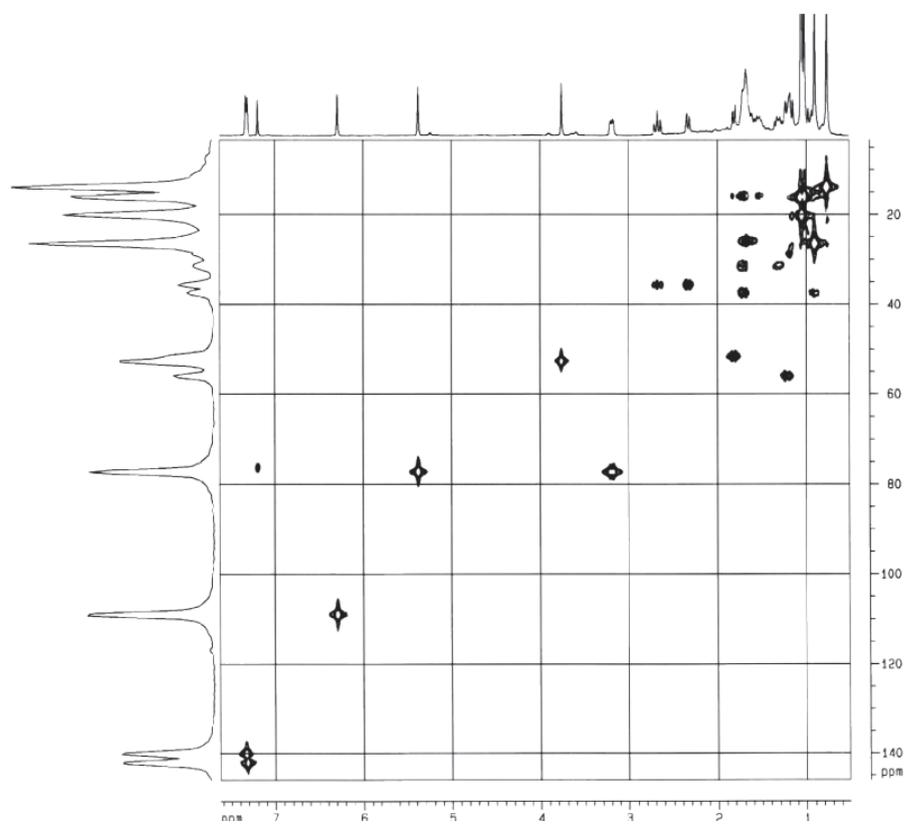
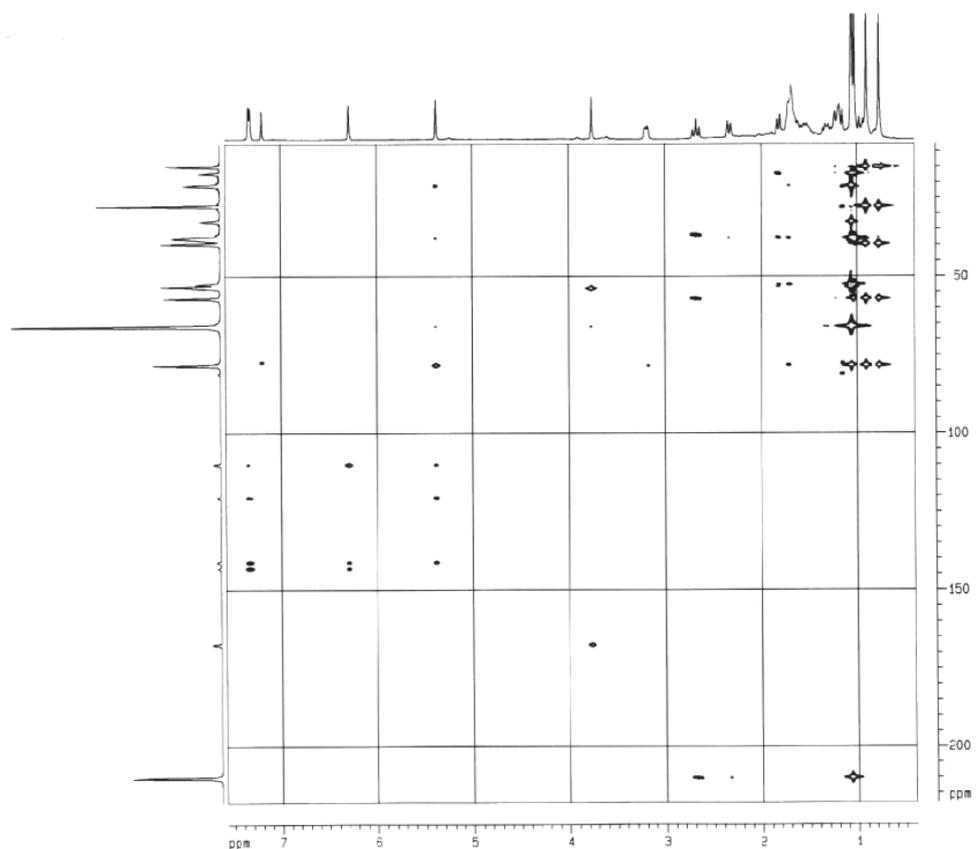
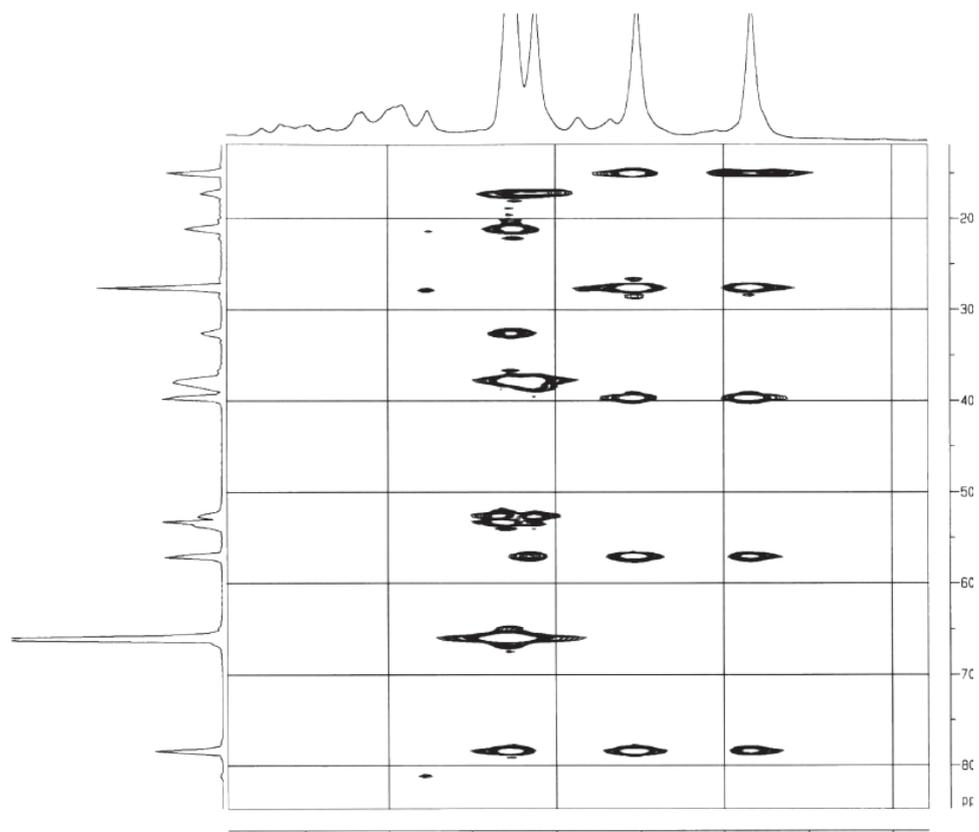


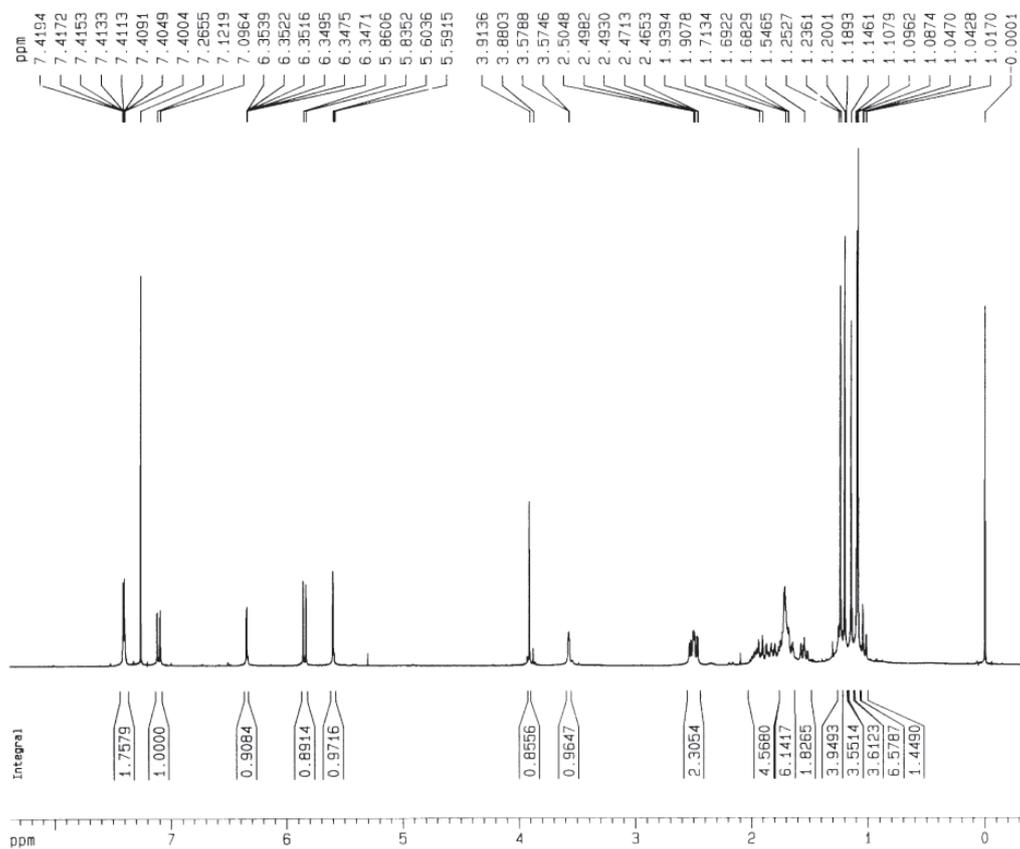
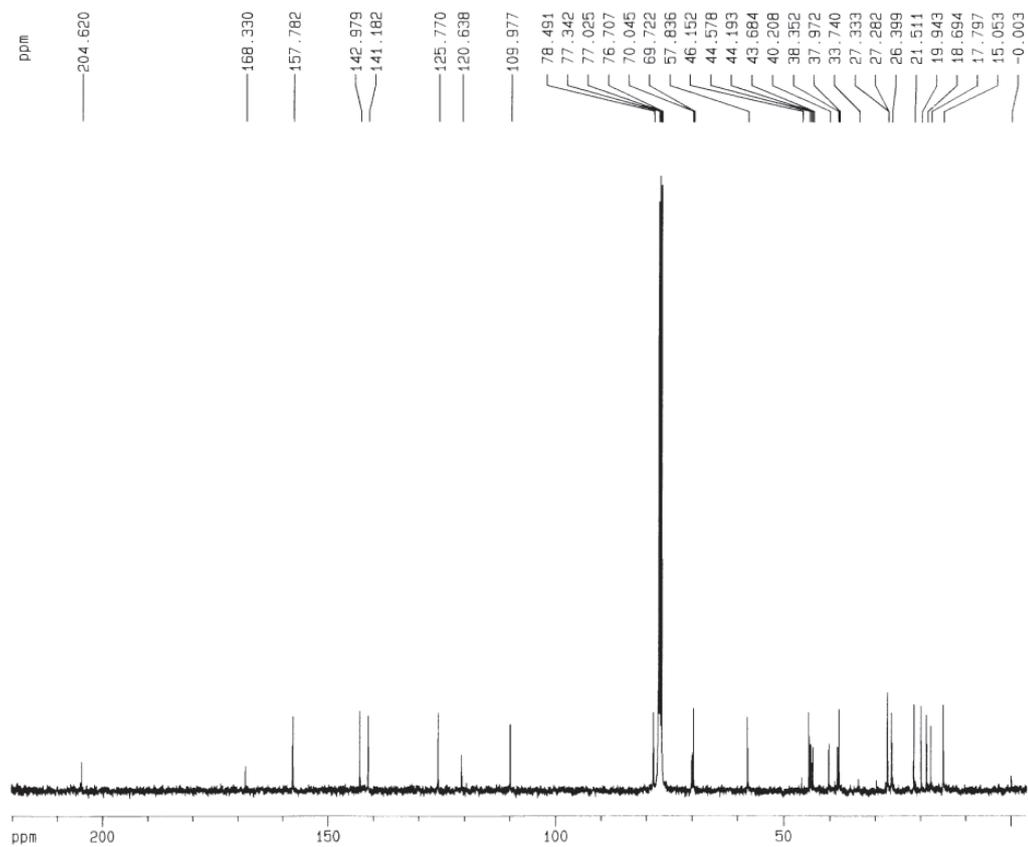
Figure S5. Correlation map HSQC of **5** (400 MHz, CDCl<sub>3</sub>).



**Figure S6.** Correlation map HMBC of **5** (400 MHz, CDCl<sub>3</sub>) A.



**Figure S7.** Correlation map HMBC of **5** (400 MHz, CDCl<sub>3</sub>) B.

Figure S8. NMR  $^1\text{H}$  spectra of **6** (400 MHz,  $\text{CDCl}_3$ ).Figure S9. NMR  $^{13}\text{C}$  spectra of **6** (100 MHz,  $\text{CDCl}_3$ ).

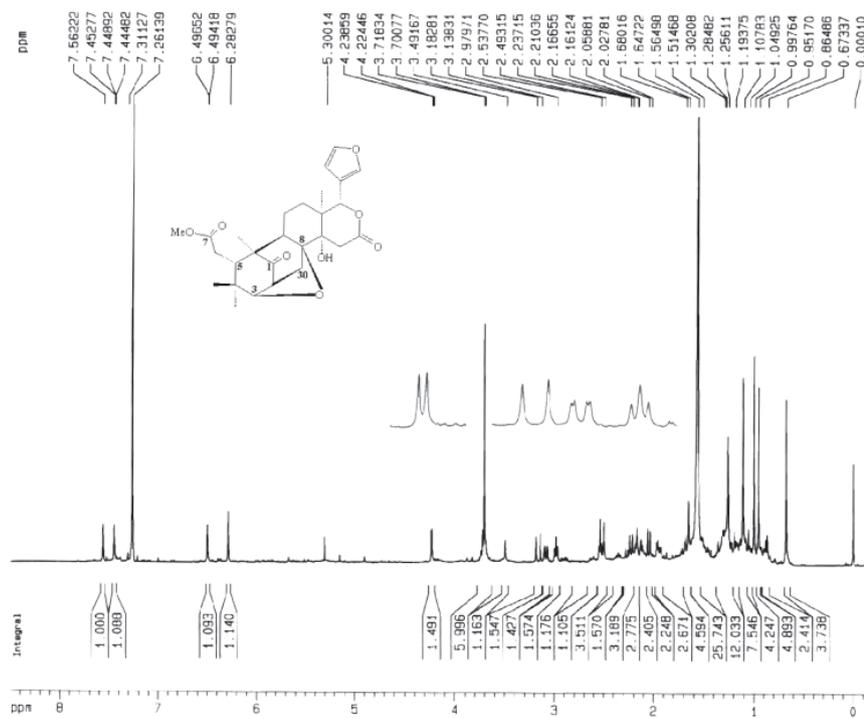


Figure S10. NMR  $^1\text{H}$  spectra of **9** (400 MHz,  $\text{CDCl}_3$ ).

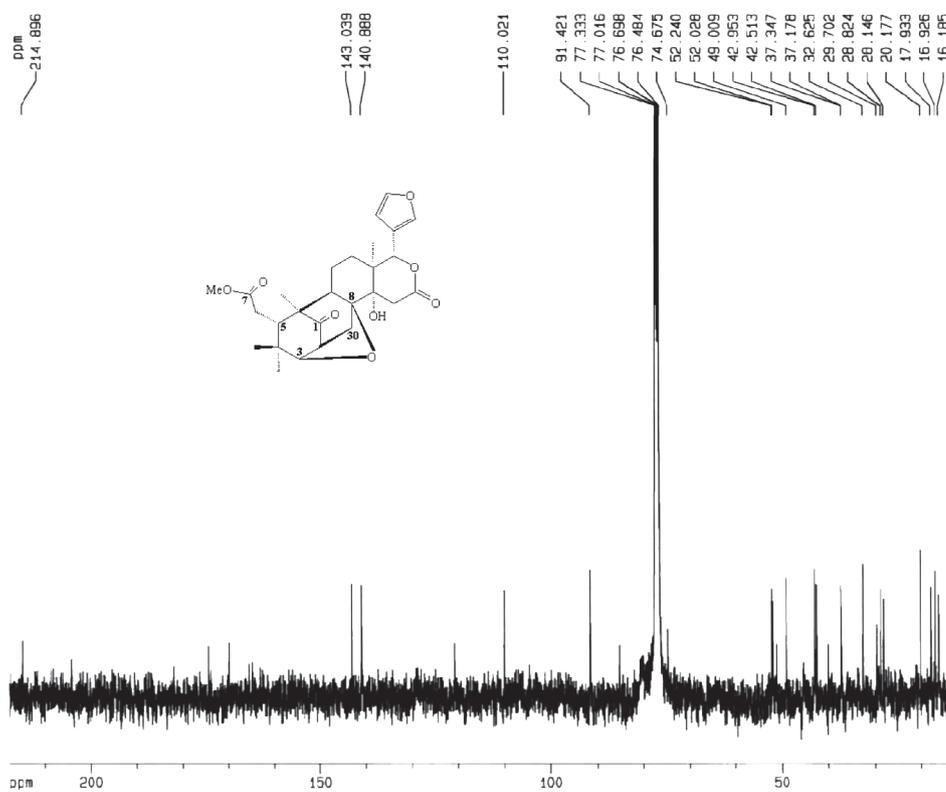


Figure S11. NMR  $^{13}\text{C}$  spectra of **9** (100 MHz,  $\text{CDCl}_3$ ).

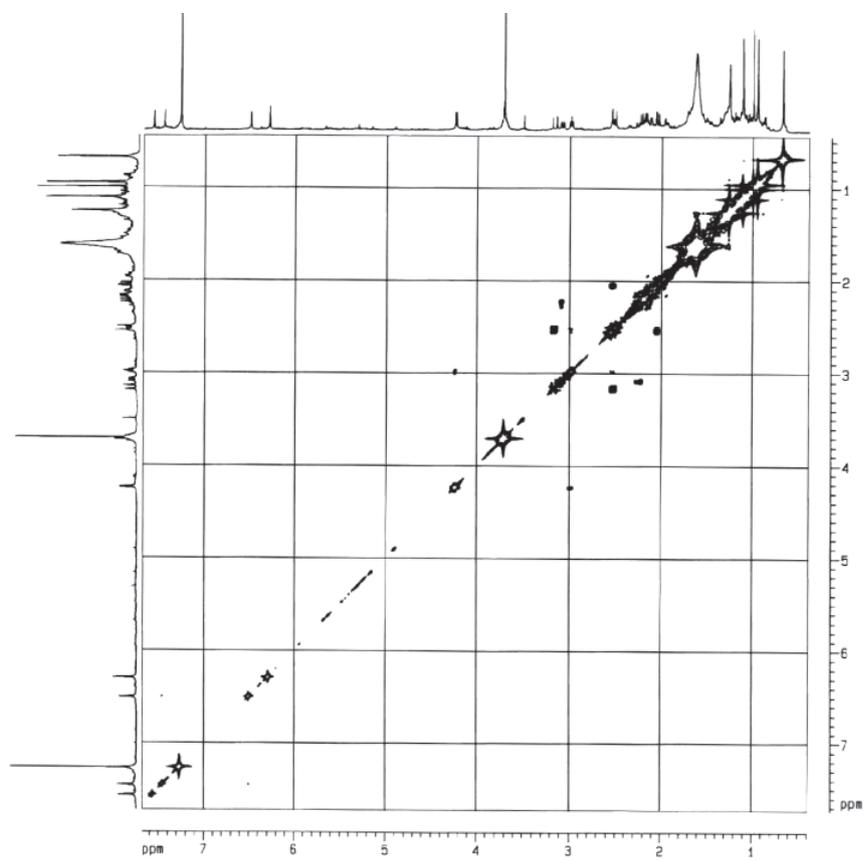


Figure S12. COSY  $^1\text{H}$ - $^1\text{H}$   $45^\circ$  spectra of **9** (400 MHz,  $\text{CDCl}_3$ ) A.

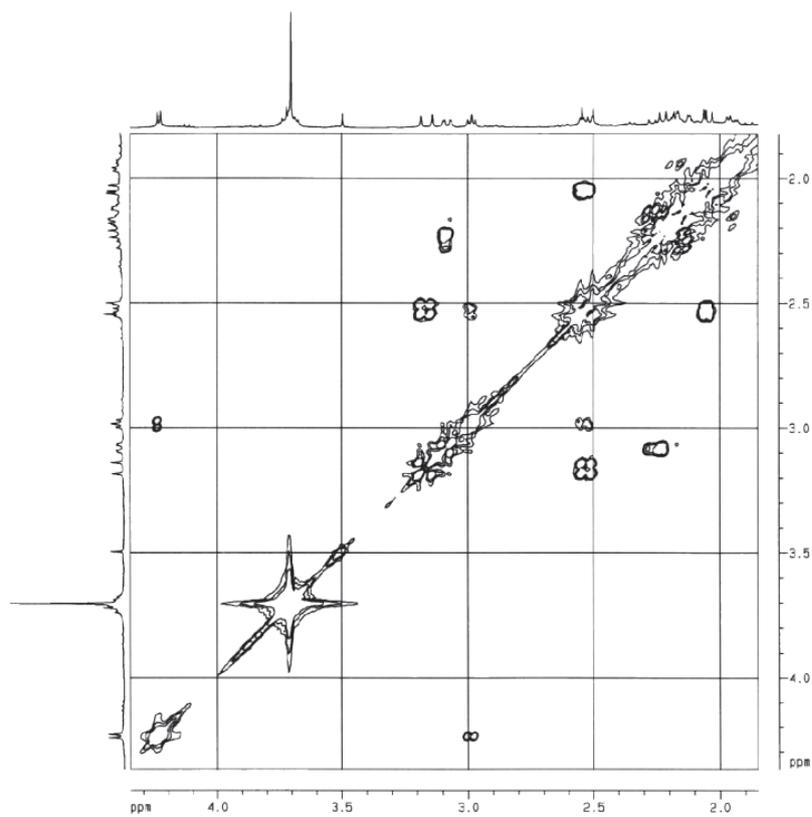
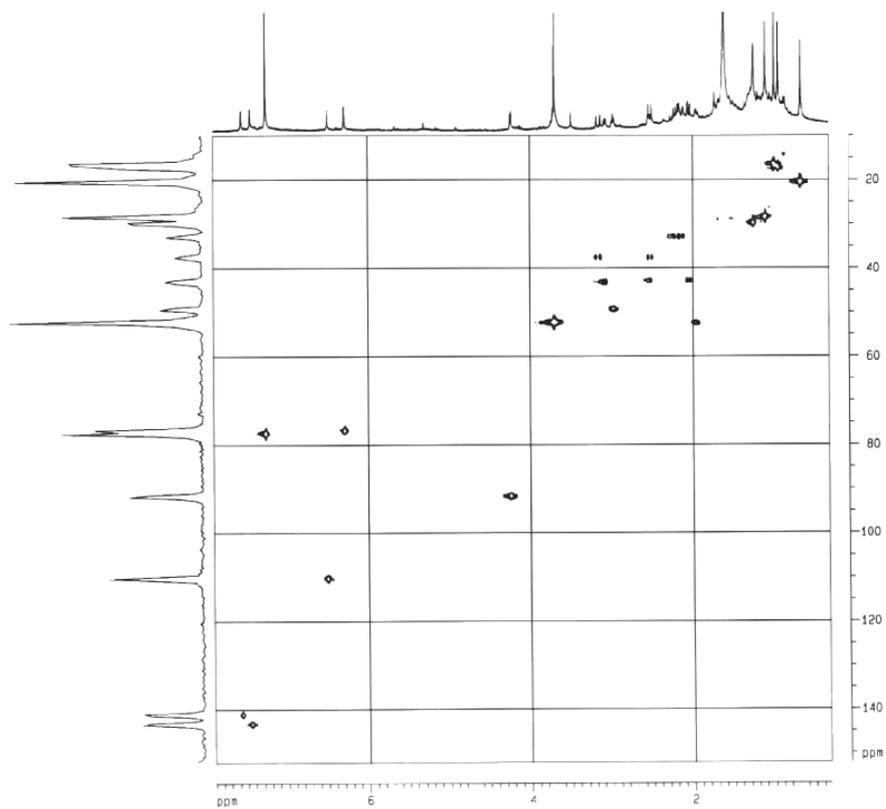
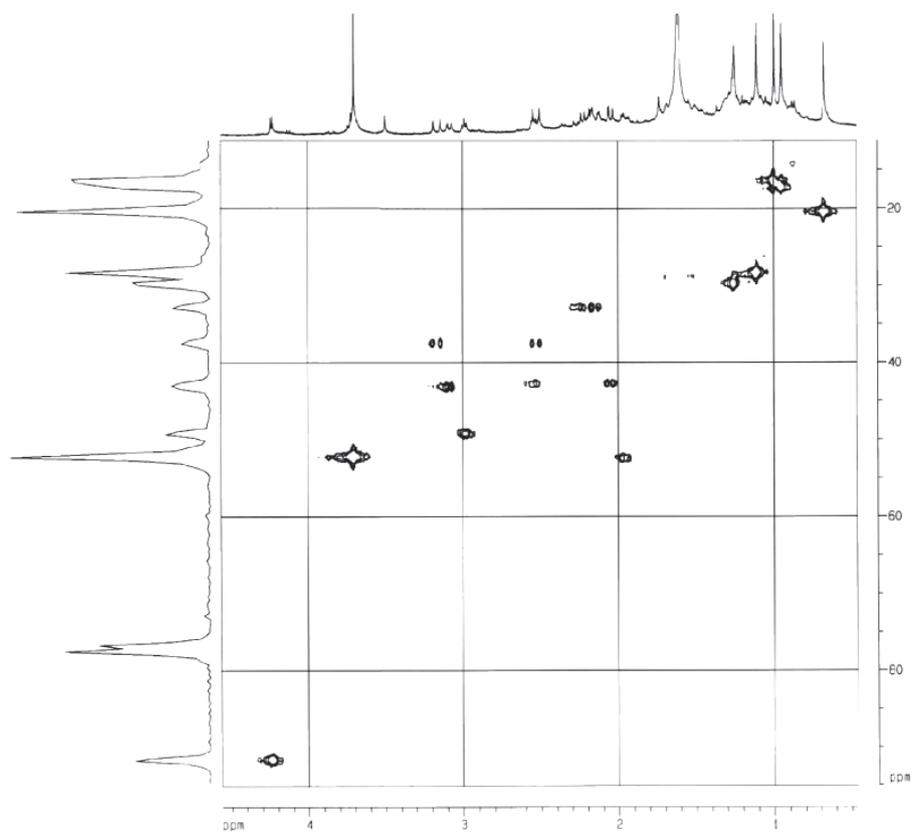


Figure S13. COSY  $^1\text{H}$ - $^1\text{H}$   $45^\circ$  spectra of **9** (400 MHz,  $\text{CDCl}_3$ ) B.



**Figure S14.** Correlation map HSQC of **9** (400 MHz, CDCl<sub>3</sub>) A.



**Figure S15.** Correlation map HSQC of **9** (400 MHz, CDCl<sub>3</sub>) B.

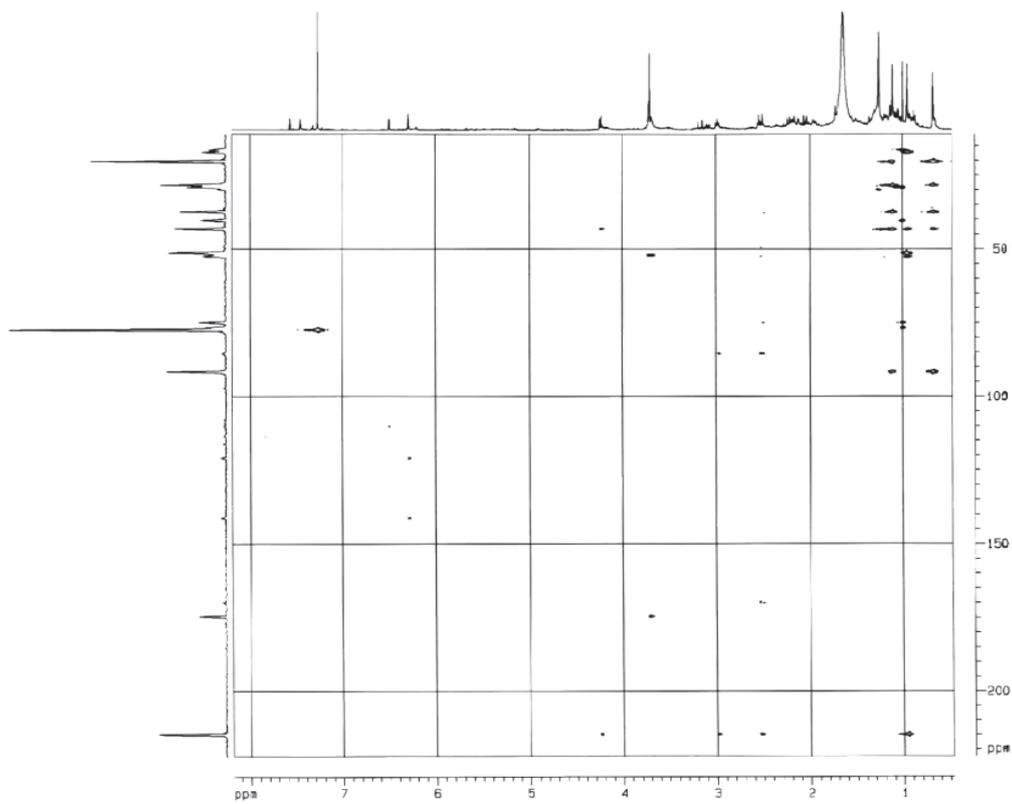


Figure S16. Correlation map HMBC of **9** (400 MHz, CDCl<sub>3</sub>) A.

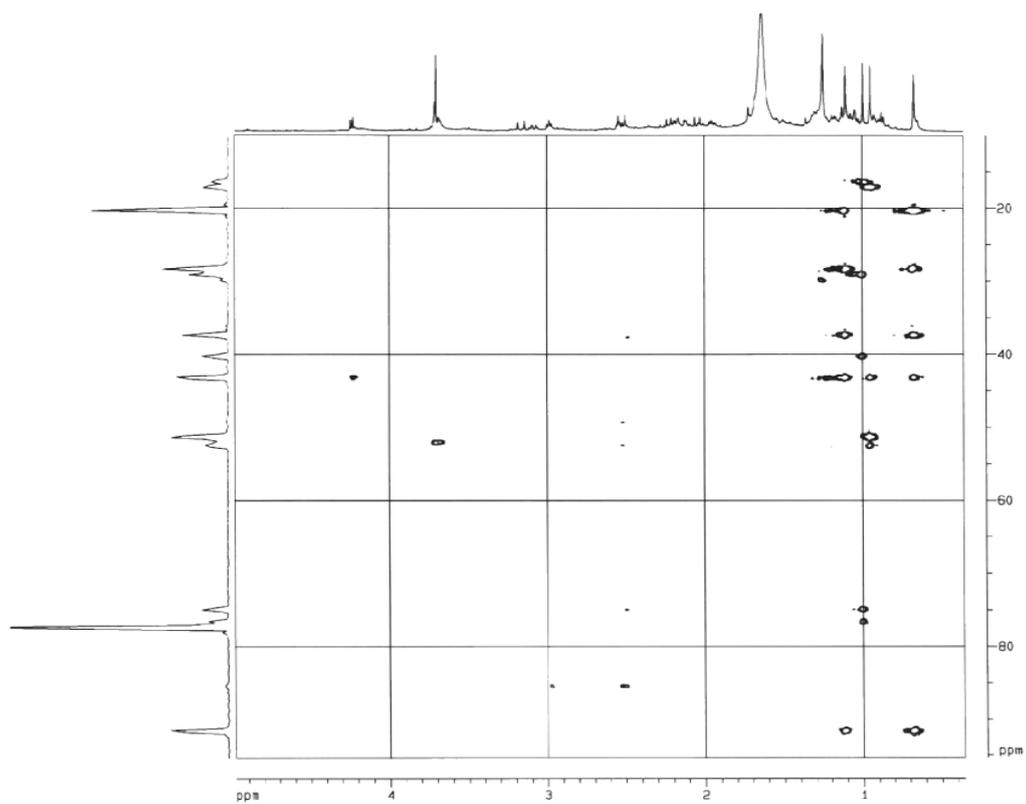


Figure S17. Correlation map HMBC of **9** (400 MHz, CDCl<sub>3</sub>) B.