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Artigo

EVALUATION OF ANTIMICROBIAL ACTIVITY AND TOXIC POTENTIAL OF EXTRACTS AND TRITERPENES ISOLATED FROM *Maytenus imbricata*

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The phytochemical study of hexane/ethyl ether (1:1) extract of the roots of *M. imbricata*, Celastraceae, resulted in the isolation and characterization of six known triterpenes: 11α-hydroxylup-20(29)-en-3-one, previously isolated from this species besides, 3β,11α-di-hydroxylup-20(29)-ene, 3,7-dioxofriedelane, 3-oxo-29-hydroxyfriedelane, tingenone and 6-oxo-tingenol. The chemical structures of these triterpenes were established by spectrometric data (IR, ^{1}H and ^{13}C NMR) and through comparison with literature data. The hexane/ethyl ether (1:1), ethyl acetate and methanol extracts, and 11α-hydroxylup-20(29)-en-3-one, tingenone and 6-oxo-tingenol, showed antimicrobial properties on *in vitro* assays. All extracts and triterpenes, except 3β,11α-di-hydroxylup-20(29)-ene, presented toxicity demonstrated by the larvicidal effect test using *Artemia salina*.

Keywords: *Maytenus imbricata*; Celastraceae; pentacyclic triterpenes.

INTRODUCTION

From different species of the genus *Maytenus*, various groups of secondary metabolites have been found, such as triterpenes,[1] sesquiterpenes,[2] phenolic glycosides,[3] alkaloids,[1] flavonoids,[4,5] and tannins,[5] among others. Members of this genus are important not only in terms of experimentally observed biological activities,[6] but also because they are used in folk medicine for gastric diseases,[7] and as antiseptic, anti-asthmatic, anti-tumor,[8] antiviral,[9] and anti-inflammatory agents.[10] In a recent review, Niero *et al.*[11] provided an adequate description of the ethnopharmacological, chemical and pharmacological knowledge about species of the genera *Maytenus*, with particular emphasis on those growing in Brazil. Many species of this genus are effective medicines, and represent promising sources of bioactive substances of medicinal interest.

The indiscriminate use of antibiotics has induced an increase in the incidence of infectious diseases caused by pathogenic microorganisms that have acquired resistance to several antibiotics currently used in clinical treatments. This scenario has led to a continuous, urgent search for new antimicrobial compounds, especially those bearing different chemical structures and having specific mechanisms of action.[12] In this context, the compounds isolated from plants have emerged as a promising alternative. Indeed, around 50% of drugs approved by the Food and Drug Administration (FDA) between 1981 and 2006 were of natural origin.[13]

It has been reported that the molecular diversity of natural products is higher than those derived from chemical synthesis processes. Thus, the biological assays of natural products represent a continuing source of new structural models for compounds with antimicrobial properties and are an important alternative for reducing the incidence of infectious diseases.[14]

According to the literature, compounds that present brine shrimp (*Artemia salina*) toxicity, in general also have cytotoxic properties against cells of solid tumors found in humans. This bioassay has being considered as adequate for initial screening of bioactive molecules, paving the way for subsequent tests of greater complexity such as *Aedes aegypti* larvicidal assays or for testing the response of cancer cells to anti-cancer drugs.[15]

Maytenus imbricata Mart, ex. Reissek is a shrub or subshrubs found in Cerrado regions (rupestrian fields) of Minas Gerais and Bahia States, Brazil. Leaves, twigs and stems of this species were subjected to biological assays. The $CHCl_3$, EtOAc and EtOH extracts of leaves, the hydroalcoholic extract of roots, the EtOAc extract of stems and epicatechin, isolated from *M. imbricata* showed antioxidant activity. The 3,4-*seco*-friedelan-3-oic acid, isolated from leaves, presented inhibitory activity of ATP synthesis raising possibilities for its potential use in the future development of natural herbicides.[16]

In this paper, the phytochemical study of the roots of *Maytenus imbricata* and isolation of the pentacyclic triterpenes (PCTT) 11α-hydroxylup-20(29)-en-3-one (**1**), 3β,11α-di-hydroxylup-20(29)-ene (**2**), 3,7-dioxofriedelane (**3**), 3-oxo-29-hydroxyfriedelane (**4**), tingenone (**5**) and 6-oxo-tingenol (**6**) (Figure 1) were reported.

The extracts, and some constituents from roots, of *M. imbricata* were submitted to *in vitro* antimicrobial assays to evaluate their properties against the bacteria *Staphylococcus aureus, Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli* and the fungus *Candida albicans*. The cytotoxic effect of these constituents was screened through their larvicidal effect on *Artemia salina*.

EXPERIMENTAL

Plant material

Roots of *Maytenus imbricata* (Celastraceae) were carefully collected so as to prevent damage to the specimen. The collection area is located on Morro de Santana, Ouro Preto municipality, Minas Gerais, Brazil. The plant material was identified by the botanists Dr. R. M. de C. Okano, Departamento de Botânica of the Universidade

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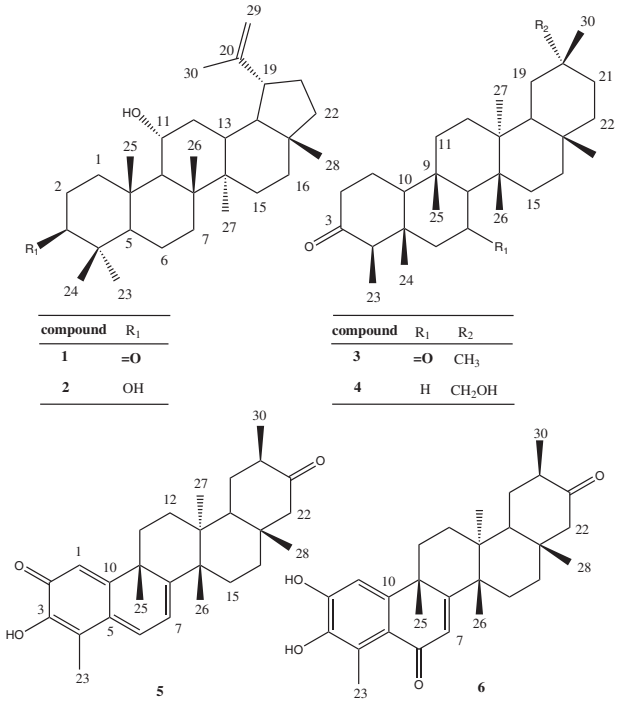

Figure 1. *Chemical structures of pentacyclic triterpenes isolated from roots of Maytenus imbricata*

Federal de Viçosa (UFV) and M. C. T B. Messias, Departamento de Botânica of the Universidade Federal de Ouro Preto (UFOP). A voucher specimen (Nº 27780) was deposited in the collection of the Herbarium of Departamento de Botânica, UFV, Brazil.

General procedures

Purification processes by column chromatography (CC) were carried out using silica gel 60 (0.063-0.200 mm) as the stationary phase. Organic solvents or mixtures of increasing polarity were used as mobile phases. Silica gel 60 GF (Merck) was used to perform analytical [(TLC), 0.25 mm] or preparative [(PTLC), 0.75 mm] thin layer chromatographic processes.

The ^{1}H (400 or 200 MHz) and ^{13}C (100 or 50 MHz_3) NMR spectra were obtained on a Bruker *Avance* DRX-400 or DPX-200 spectrometer, operating at 300 K. The chemical shifts (δ) were expressed in units of ppm, using TMS as a reference ($\delta_H = \delta_C = 0$) and the coupling constants (*J*) were expressed in Hz. $CDCl_3$ was used as the solvent for the samples.

The IR spectra of constituents in KBr discs were obtained on a Shimadzu IR408 spectrometer and the results reported in reciprocal centimeters (cm^{-1}) in the range 400-4000 cm^{-1}.

The melting point (mp) ranges were determined on an MQAPF-302 apparatus (Microquímica Equipamentos Ltda, Brazil).

Extraction and isolation of compounds

The collected roots of *M. imbricata* were powdered in a mill. The powder (1.5 kg) was then submitted to extractions in a Soxhlet apparatus with hexane-ethyl ether (1:1), ethyl acetate, and finally with methanol. During extraction with hexane/ethyl ether (1:1) a solid was formed and separated by filtration giving the hexane/ethyl ether solid material [HES (22.2 g)]. The solvent of the filtrated product was removed in a rotatory evaporator, giving the hexane/ethyl ether (1:1) extract [HEE (16.1 g)]. During extraction using ethyl acetate, another solid was formed and separated by filtration, giving the ethyl acetate solid [EAS (56.2 g)]. The resultant product of solvent removal was ethyl acetate extract [EAE (21.2 g)]. Finally, the methanol extract [ME (176.7 g)] was obtained. A total of 2 L of hexane/ethyl ether (1:1), 2 L of ethyl acetate and 2 L of methanol were used in the extraction processes.

The extract HEE (3.0 g) was submitted to silica gel (300.8 g) CC eluted with hexane-EtOAc. Three hundred fifty fractions of 100 mL each were obtained and grouped according to the similar profiles observed in the chromatoplates. Fractions 14 to 19 produced a white crystalline solid (12.3 mg), 0.41% yield and mp 247.7-250.1 °C. This solid was identified as 3,7-dioxo-friedelane (**3**). The fractions 44-49 provided a white solid in the form of flakes (63.1 mg), 2.10% yield, mp 154.2-158.8 °C, subsequently identified as 11α-hydroxylup-20(29)-en-3-one (**1**). Fractions 84-95 produced a white crystalline solid (7.0 mg), 0.23% yield, mp 146.9-148.4 °C which was identified as 3-oxo-29-hydroxyfriedelane (**4**). Fractions 107-128 produced an amorphous orange solid (183.3 mg) which was subjected to silica gel CC eluted with Hex, EtOAc and MeOH, pure or in mixtures of increasing polarity, providing 240 fractions of 10 mL each. Solvent evaporation from fractions 211 to 235 (eluted with Hex/EtOAc 6:4) produced an orange solid (64.4 mg), 2.15% yield, mp 145.0-147.9 °C, which was identified as tingenone (**5**).

Fractions 205-207 produced an amorphous orange solid (146.7 mg) which was submitted to silica gel CC (47.3 mg) eluted with Hex, EtOAc and MeOH, pure or in mixtures of increasing polarity, providing 137 fractions of 10 mL each. After the solvent was withdrawn, fractions 1 to 15 (eluted with Hex/EtOAc 9:1) yielded an orange solid (25.3 mg), which showed three spots when analyzed by TLC. These fractions (1-15) were submitted to silica gel PTLC eluted with Hex/EtOAc 7:3. The less polar compound (Rf ~ 0.9) was isolated as a white solid (11.0 mg), 0.37% yield, mp 212.9-218.6 °C and was identified as 3β,11α-di-hydroxylup-20(29)-ene (**2**). Fraction 76 yielded a light yellow solid (8.0 mg), 0.27% yield, mp 218.7-221.4 °C that was identified as 6-oxo-tingenol (**6**). These terpenes were characterized through their NMR spectral data and also by comparing with published data.

The extract HES (2.0 g) was submitted to silica gel (107.9 g) CC eluted with Hex, EtOAc and MeOH, pure or in mixtures of increasing polarity, providing 114 fractions of 25 mL each that were grouped according to the similar profiles observed in the chromatoplates.

Fractions 33-42 produced an amorphous brown solid (55.0 mg) which was subjected to silica gel CC eluted with Hex, EtOAc and MeOH, pure or in mixtures of increasing polarity, providing 117 fractions of 3 mL each. After solvent evaporation from fractions 33 to 47 (eluted with Hex/EtOAc 2:8), a white solid in the form of flakes was obtained (14.0 mg), 0.7% yield and mp 154.2-158.8 °C. This solid was identified as 11α-hydroxylup-20(29)-en-3-one (**1**).

Fractions 66-85 produced an orange solid (314.0 mg), 15.7% yield, mp 145.0-147.9 °C, which was identified as tingenone (**5**).

Fractions 94-102 produced an amorphous brown solid (300.9 mg) which was subjected to silica gel CC eluted with Hex, EtOAc and MeOH, pure or in mixtures of increasing polarity, providing 294 fractions of 3 mL each. Solvent evaporation from fractions 121 to 133 (eluted with EtOAc) produced a light yellow solid (7.9 mg), 0.4% yield, mp 218.7-221.4 °C which was identified as 6-oxo-tingenol (**6**).

The extracts EAE, EAS and ME have yet to be submitted to phytochemical studies. However phytochemical prospection[17] allowed the detection of the presence of alkaloids, flavonoids and catechin.

Biological assays

Antimicrobial activity

To evaluate antibacterial and antifungal activity, the microdilution

method was used to determine the average minimal inhibitory concentrations inhibiting the growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the microorganisms.[18] All extracts and the triterpenes **1**, **5** and **6** were tested against *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 25723), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and *Candida albicans* (ATCC 18804). The bacteria and the fungus were maintained in brain heart infusion (BHI) culture medium, at 7 ºC. An initial duplicate screening was carried out using disposable microplates with all samples being tested at a concentration of 100.00 µg/mL. In this step, all extracts and the triterpenes **1**, **5** and **6** showed antimicrobial activity. Subsequently, these were tested at concentrations of 250.00, 125.00, 62.50, 31.30, 15.60, 7.81, 3.91, 1.95, 0.98, 0.49, 0.24 and 0.12 µg/mL to determine the minimal inhibitory concentration (MIC_{50} and MIC_{90}). All 12 microdilution assays were performed in duplicate. Chloramphenicol (MIC_{50} = 0.24 µg/mL and MIC_{90} = 15.60 µg/mL) was used as a positive control for bacteria and miconazole (MIC_{50} = 3.32 µg/mL and MIC_{90} = 600.48 µg/mL) for *C. albicans*. The inocula of bacteria and fungus used in experiments contained 4.16 x 10^3 cells/mL. At the end of incubation time (24 h), the plates were analyzed using a Microplate TP-Reader (Thermoplate, Brazil).

Evaluation of toxic potential through *Artemia salina* larvicidal activity

The cysts of *Artemia salina* for the test were acquired in Belo Horizonte City, MG, Brazil. The *A. salina* cysts (10 mg) were added to 100 mL of synthetic marine salt solution (38.0 g/L) previously prepared using deionized water, and maintained under artificial light at 28 °C. Larvae hatching occurred after 24 h of incubation time. Lapachol, a compound with proven anti-*A. salina* effect, was used as the control. The extracts and terpenes **1**, **3**, **4**, **5** and **6** isolated from *M. imbricata* were submitted to larvicidal assays, at concentrations ranging from 1000.00 to 0.06 µg/mL, to determine their toxic potential. The concentration range was chosen based on the highest concentration that showed 100% mortality and at the concentration which induced no death of *A. salina*. The samples were dissolved in DMSO and their concentration ranges were HES and HEE (15.62-0.48 µg/mL), EAS, EAE and ME (1,000.00-62.50 µg/mL), terpene **1** (31.25-1.95 µg/mL), **3** and **4** (125.00-7.81 µg/mL), **5** (0.97-0.06 µg/mL) and **6** (500.00-31.25 µg/mL). Terpene **2** was not submitted to larvicidal assays against *A. salina* because it was used in another test and an insufficient amount remained for testing.

RESULTS AND DISCUSSION

Phytochemical study

Through the phytochemical study of HEE it was possible to isolate the known pentacyclic triterpenes **1**, **2**, **3**, **4**, **5** and **6** (Figure 1). The chemical structures of these constituents were identified based on IR, ^{1}H and ^{13}C NMR spectral data, and by comparing with literature data.

Compound **1** was previously isolated through phytochemical study of the aerial part of *M. imbricata*.[19] In this paper, the isolation of terpenes **2**, **3**, **4**, **5** and **6** from *M. imbricata* is reported for the first time.

The profile of IR spectra of compounds **1** to **5** were in accordance with the published data for pentacyclic triterpenes.[20-23]

The ^{1}H NMR spectrum of **1** disclosed signals of 7 methyl groups [δ 1.69 (3H), δ 1.09 (3H), δ 1.07 (6H), δ 1.06 (3H), δ 0.98 (3H) and δ 0.80 (3H)], of methine [triple doublet at δ 3.91 ($H11_{ax\text{-}ax}$, J = 10.8 Hz; and $H11_{ax\text{-}eq}$, J = 4.8 Hz)] and of olefin hydrogens (δ 4.72 and δ 4.60). These data, together with the signals at δ 109.95 (C29) and δ 150.22 (C20) observed in the ^{13}C NMR spectrum, suggested that **1** was a PCTT of lupane series,[21] which was then compared with the literature.[22]

Seven methyl signals [δ 1.69 (3H), δ 1.26 (3H), δ 1.04 (9H), δ 0.96 (3H) and δ 0.79 (3H)], together with the signals of methine hydrogen [δ 3.93 ($H11_{ax\text{-}ax}$, J = 10.8 Hz and $H11_{ax\text{-}eq}$, J = 4.8 Hz) and of methine hydrogen H3 (δ 3.21), were observed in the ^{1}H NMR spectrum of **2**.[21] Two singlet signals (δ 4.72 and δ 4.59) were attributed to H29 of terminal double bond. The signals of carbons [δ 109.95 (C29) and δ 150.25 (C20)] confirmed the double bond and suggested **2** as member of the PCTT lupane series.[21] The NMR spectral data of **2** were in accordance with literature data.[21]

The signals in the ^{1}H NMR spectrum of **3** at δ 0.77; δ 0.88; δ 0.91; δ 0.96; δ 1.00; δ 1.07 and δ 1.18 were attributed to seven methyl groups, and a doublet at δ 0.88 was associated to another methyl group (C23). These signals are commonly related to PCTT of the friedelane series.[21] The ^{13}C NMR spectrum of **3** revealed signals at δ 211.12 and at δ 210.62 corresponding to ketone carbonyls. The chemical shift values found in the ^{13}C NMR spectrum of terpene **3** were compared with literature data.[21]

The signals at δ 0.73; δ 0.87; δ 1.03; δ 1.04; δ 1.05 and δ 1.22 observed in the ^{1}H NMR spectrum of **4** were associated to 6 methyl groups, and a doublet signal at δ 0.88 to another methyl group (C23), while a doublet at δ 3.27 was attributed to hydrogen linked to hydroxylated carbon. The ^{13}C NMR spectrum disclosed signals at δ 6.83 assigned to methyl carbon (C23) of PCTT friedelane,[21] at δ 213.18 attributed to carbonyl (C3) and at δ 74.78 assigned to hydroxylated carbon (C29). The NMR data of terpene **4** were in accordance with published data.[21]

The profile of the IR spectrum, especially in the region between 1500-1708 cm^{-1}, characteristic of C=O and C=C bonds, suggested compound **5** as a quinone methide PCTT.[20] The signals observed in the ^{1}H NMR spectrum of **5** at δ 0.98; δ 1.01; δ 1.35; δ 1.51 and at δ 2.23 corresponded to 5 methyl groups, a doublet signal at δ 1.00 was attributed to another methyl group (C30); and a doublet at δ 2.92 (H22) and a multiplet at δ 2.51 were associated to hydrogen linked to C20. These spectral data, together with the doublet signal at δ 7.03 and at δ 6.38; and a singlet at δ 6.55, confirmed **5** as being a quinone methide triterpene.[21] The carbon signals at δ 178.43 (C2) and at δ 213.58 (C21) were attributed to carbonyl carbons. The chemical shift values found in the ^{13}C NMR spectrum of terpene **5** were consistent with the literature data.[23]

The IR spectrum of **6** revealed bands at 1592 and 1458 cm^{-1} that were associated to the C=C bond of aromatic compound.[20] Six methyl signals [singlets at δ 2.63; δ 1.58; δ 1.38; δ 1.00 and δ 0.99, as well as a doublet at δ 0.99 (C30)], were identified in the ^{1}H NMR spectrum of **6**. Two singlets, at δ 6.86 (H1) and at δ 6.25 (H7), confirmed the aromatic and olefin hydrogens, respectively. The carbon signals at δ 187.94 (C6) and at δ 214.69 (C21) were associated to carbonyls. The chemical shift values found in the ^{13}C NMR spectrum of terpene **6** were consistent with the literature data.[24]

Antimicrobial activity

The level of resistance or sensitivity of the bacteria and fungus to the samples was determined by the presence or absence of growth. The results of MIC_{50} of the extracts and triterpenes from *M. imbricata* subjected to antimicrobial assays are shown in Table 1.

In accordance with the results, it was found that all samples showed MIC_{50} within the concentration range used in the experiments. The solid HES, extract HEE, and terpene **5** showed better inhibition of *S. aureus* growth. Azithromycin is a macrolide antibiotic that suppresses the biosynthesis of protein, retards bacterial growth, or causes death of microorganisms, when tested against *S. aureus* presented a MIC_{50} of 4.0 µg/mL.[25] This represents a lower activity

Table 1. Minimal inhibitory concentrations that inhibit 50% of the microorganism growth (MIC_{50}) determined for the extracts and compounds isolated from *M. imbricata* against pathogenic microorganisms

Extracts and compounds	Minimal Inhibitory Concentration (MIC_{50}) (μg/mL)				
	S. typhimurium	*E. coli*	*S. aureus*	*B. cereus*	*C. albicans*
HES	125.00	125.00	0.12	125.00	0.49
HEE	125.00	125.00	0.12	250.00	0.12
EAS	125.00	125.00	31.30	125.00	62.50
EAE	250.00	250.00	31.30	125.00	31.30
ME	125.00	250.00	31.30	125.00	15.60
1	250.00	250.00	62.50	250.00	125.00
5	62.50	125.00	0.12	125.00	0.12
6	31.30	125.00	62.50	250.00	62.50
chloranfenicol	0.24	0.24	0.24	0.24	-
miconazole	-	-	-	-	3.32

than that found for HES, HEE, and for terpene **5**, which showed a lower value for MIC_{50}. All extracts and terpenes **5** and **6** induced a high rate of growth inhibition of *C. albicans* (Table 1). Fluconazole is an antifungal used to treat infectious diseases caused by fungus. In assays with *C. albicans*, fluconazole showed a MIC_{50} of 1.1 μg/mL.[26] The MIC_{50} value found using HES, HEE and terpene **5**, indicated its higher activity against *C. albicans*, evidencing their potential as antifungal agents.

The extract from leaves of *Maytenus ilicifolia* showed no activity against *Salmonella sp.*[27] However, in this work, it was verified that all extracts as well as the compounds **1**, **5** and **6**, obtained from roots of *M. imbricata* were active against *Salmonella typhimurium*.

As shown in Table 2, higher antimicrobial activity was found for the extract HEE against *S. aureus* (MIC_{90} = 0.12 μg/mL) and for ME against *C. albicans* (MIC_{90} = 31.30 μg/mL).

Vancomycin, an antibiotic highly effective against *Gram*-positive bacteria, has being adopted as a first choice for the treatments of infectious diseases caused by *S. aureus*. This is the main pathogen associated to hospital-acquired infections. In assays *in vitro* against *S. aureus*, vancomycin presented a value higher (MIC_{100} = 1.5 μg/mL)[28] value than that found for HEE (MIC_{90} = 0.12 μg/mL).

Neomycin is an antibiotic used to prevent or treat skin infections caused by bacteria, including *S. aureus*. Jain *et al*.[29] tested neomycin against *S. aureus*, and found MIC_{100} = 190.0 μg/mL, a value higher than the MIC_{90} of HES, HEE and compound **5.** These authors also tested the ethanol extract from roots of *Maytenus senegalensis* against *S. aureus* and found MIC_{100} = 1250.0 μg/mL. This value is higher than the MIC_{90} found for the extracts HES, HEE, EAS and for terpenes **1**, **5** and **6** (Table 2).

The methanol extract (ME) was active against *B. cereus*. This bacterium showed high sensitivity when treated with ME extract. In addition, this extract also presented activity against *E. coli* and *C. albicans*.

Considering the results of the *in vitro* assays with bacteria and *Candida albicans*, a high antibacterial and antifungal activity was attributed to the extracts and terpenes isolated from roots of *M. imbricata*.

Evaluation of cytotoxic potential

The toxicity assays using *Artemia salina*, a marine microcrustacean, were carried out according to the methodology described by Pimenta *et al.*.[30] The experiments were performed in triplicate and the lethal concentration of sample necessary to induce 50% death (LC_{50}) of brine shrimps, was established using the Probit Method, a parametric statistical procedure with a 95% confidence interval.

Table 2. Minimal inhibitory concentrations that inhibit 90% of the microorganism growth (MIC_{90}) determined for the extracts and compounds isolated from *M. imbricata* against pathogenic microorganisms

Extracts and compounds	Minimal Inhibitory Concentration (MIC_{90}) (μg/mL)				
	S. typhimurium	*E. coli*	*S. aureus*	*B. cereus*	*C. albicans*
HES	ND	ND	125.00	ND	ND
HEE	250.00	ND	0.12	ND	62.50
EAS	ND	ND	250.00	ND	125.00
EAE	ND	ND	ND	ND	250.00
ME	ND	250.00	ND	250.00	31.30
1	ND	ND	250.00	ND	250.00
5	125.00	125.00	125.00	ND	125.00
6	ND	ND	250.00	ND	ND
chloranfenicol	15.6	15.6	15.6	15.6	-
miconazole	-	-	-	-	600.48

ND = Not Detected

Extracts of plants or compounds submitted to assays with *A salina* are considered active when the LC_{50} is less than 1000.0 μg/mL.[31] The evaluation of toxicity using *A. salina* was performed with the extracts HES, HEE, EAS, EAE and ME, and compounds **1**, **3**, **4**, **5** and **6**, followed by the respective determination of LC_{50} (Table 3).

Table 3. Average lethal dose (LC_{50}) of extracts and terpenes isolated from roots of *Maytenus imbricata* against *A. salina*

Extracts and compounds	Toxicity against *A. salina*	
	LC_{50} (μg/mL)	Confidence limit (95%)
HES	2.10	$1.66 < LC_{50} < 2.65$
HEE	2.53	$0.97 < LC_{50} < 3.09$
EAS	236.16	$197.92 < LC_{50} < 281.79$
EAE	295.87	$244.36 < LC_{50} < 358.24$
ME	384.93	$308.47 < LC_{50} < 480.34$
1	9.28	$7.69 < LC_{50} < 11.20$
3	26.05	$19.60 < LC_{50} < 34.62$
4	47.43	$39.83 < LC_{50} < 56.48$
5	0.15	$0.11 < LC_{50} < 0.19$
6	33.74	$26.13 < LC_{50} < 43.58$
lapachol	70.00	$61.00 < LC_{50} < 81.00$

LC_{50} = concentration that causes 50% death.

The extracts HES and HEE and all terpenes showed high toxicity, evidenced by their low LC_{50} values (Table 3). Tingenone (**5**) is a pentacyclic triterpene with known cytotoxic properties.[32] In the present work, this terpene showed higher toxicity when compared to the other samples of *M. imbricata* tested. This terpene is the main constituent found in the extracts HES (40%) and HEE (9.4%). Thus, it is possible to assign the high cytotoxicity observed for these two extracts to compound **5**. Using *Artemia franciscana*, Macari *et al.*[33] studying *Maytenus guyanensis* found LC_{50} = 363 μg/mL for the hexane extract of barks. Based on this LC_{50}, these authors considered this extract as being a potential larvicide product. Through the assay with *A. salina*, it was found that, except for the extract ME, all other samples presented values of LC_{50} lower than 363 μg/mL. Based on these results can be established that substances from *M. imbricata* also represent potential larvicide agents. Bouzada *et al.*,[34] using a similar methodology with *A. salina*, found $LC_{50} > 250$ μg/mL for the methanol extract from leaves of *Maytenus ilicifolia*. On the other hand, ethanol extract of *Maytenus obtusifolia* was considered of low toxicity to the larvae of *A. salina*, with LC_{50} greater than 1000 mg/mL.[35] The values of this average lethal dose (LC_{50}) are high when compared with those found for HES and HEE in the present work (Table 3). Since the extracts and terpenes showed good activity against *A. salina* it is possible to conclude that they also have potential antitumor, pesticide, trypanosomicide and/or molluscicide activities.[15]

CONCLUSION

The triterpenes 11α-hydroxylup-20(29)-en-3-one, 3β,11α-dihydroxylup-20(29)-ene, 3,7-dioxofriedelane, 3-oxo-29-hydroxyfriedelane, tingenone and 6-oxo-tingenol were isolated from roots of *Maytenus imbricata*. All extracts, and the terpenes 11α-hydroxylup-20(29)-en-3-one, tingenone and 6-oxo-tingenol, were active against *S. typhimurium, E. coli, S. aureus, B. cereus* and *C. albicans*. The extracts HES and HEE and the terpenes **1**, **3**, **4**, **5** and **6** showed representative larvicidal effects against *Artemia salina*. The results of this work indicate the promising potential of this plant as a larvicide, or as a source of drugs with antimicrobial or antitumor properties.

SUPPLEMENTARY MATERIAL

The Figures 1S-21S present spectra of IR, ^{1}H and ^{13}C NMR of compounds isolated of hexane/ethyl ether (1:1) extract of the roots of *M. imbricata*. The Tables 1S-6S present comparison of ^{13}C NMR data of compounds and of literature. This supplementary material is available free of charge at http://quimicanova.sbq.org.br, as PDF file.

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EVALUATION OF ANTIMICROBIAL ACTIVITY AND TOXIC POTENTIAL OF EXTRACTS AND TRITERPENES ISOLATED FROM *Maytenus imbricata*

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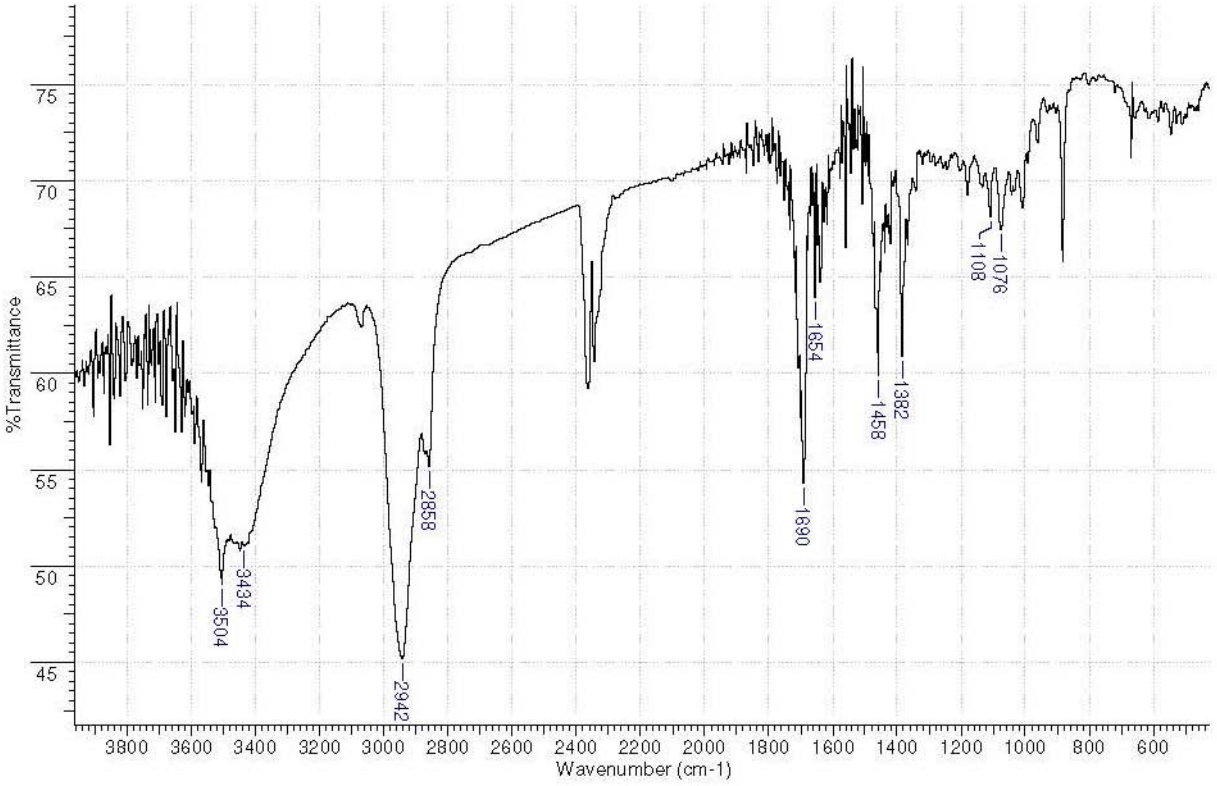

Figure 1S. *IR spectrum of compound* ***1*** *(KBr, cm^{-1})*

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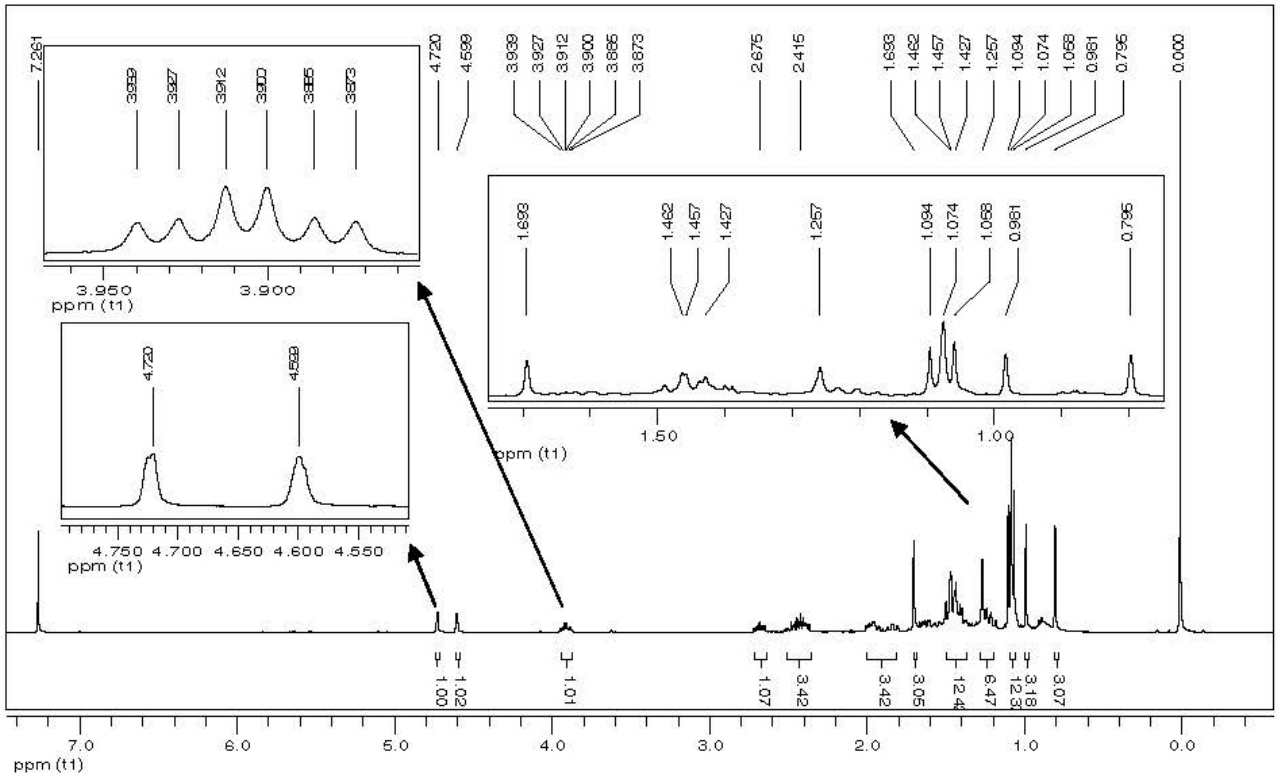

Figure 2S. *^{1}H NMR spectrum of compound* ***1*** *(400 MHz, $CDCl_3$)*

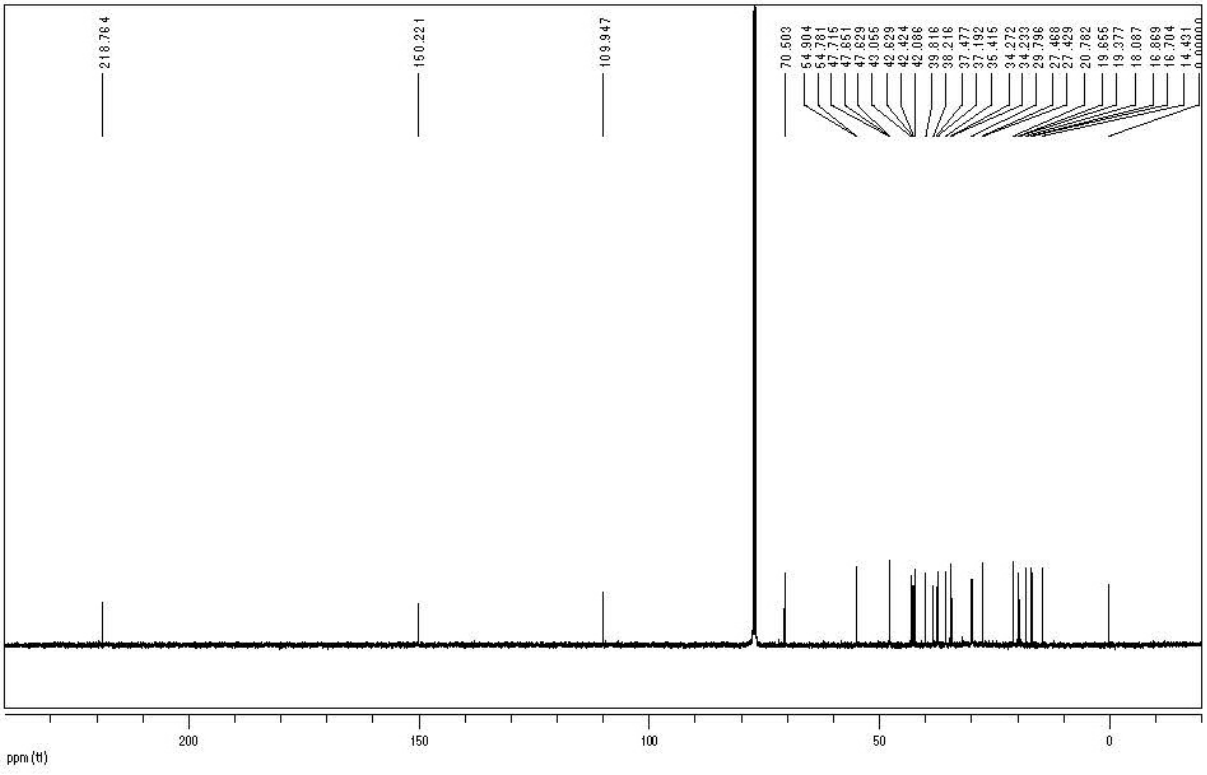

Figure 3S. *^{13}C NMR spectrum of compound* ***1*** *(100 MHz, $CDCl_3$)*

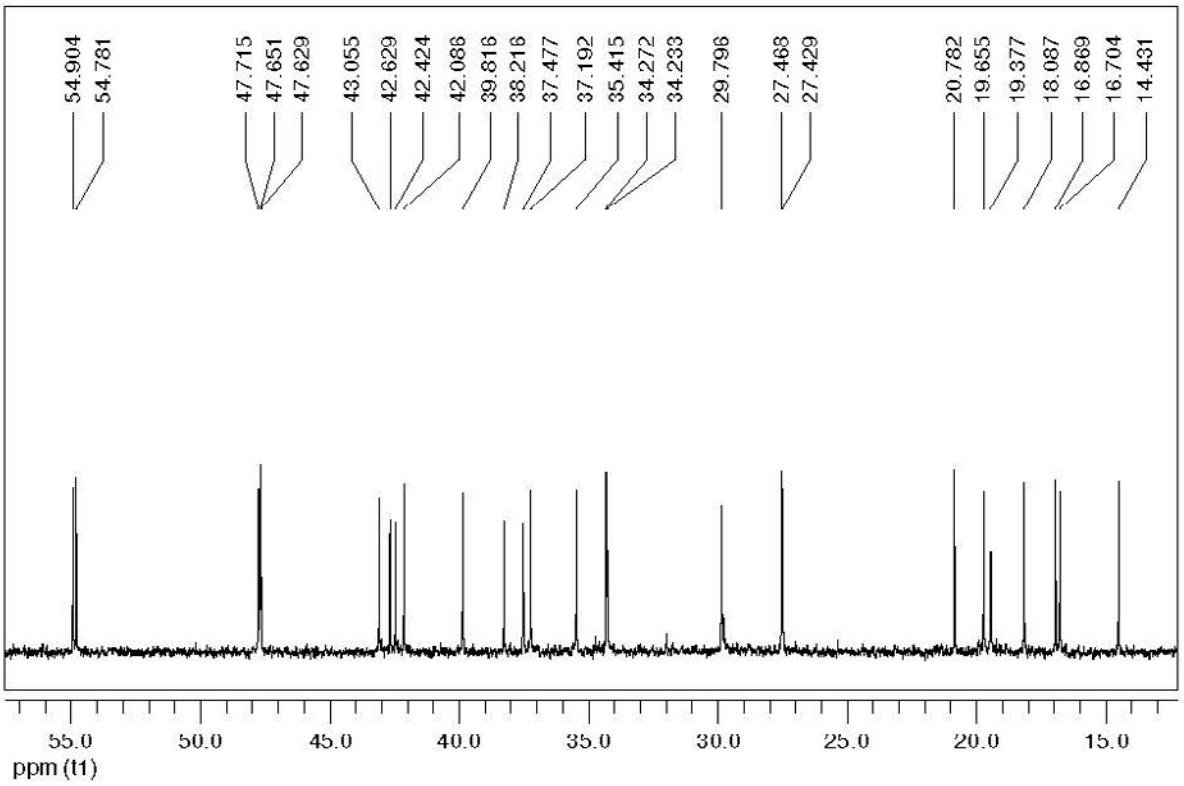

Figure 4S. *^{13}C NMR spectrum of compound **1** (100 MHz, $CDCl_3$)*

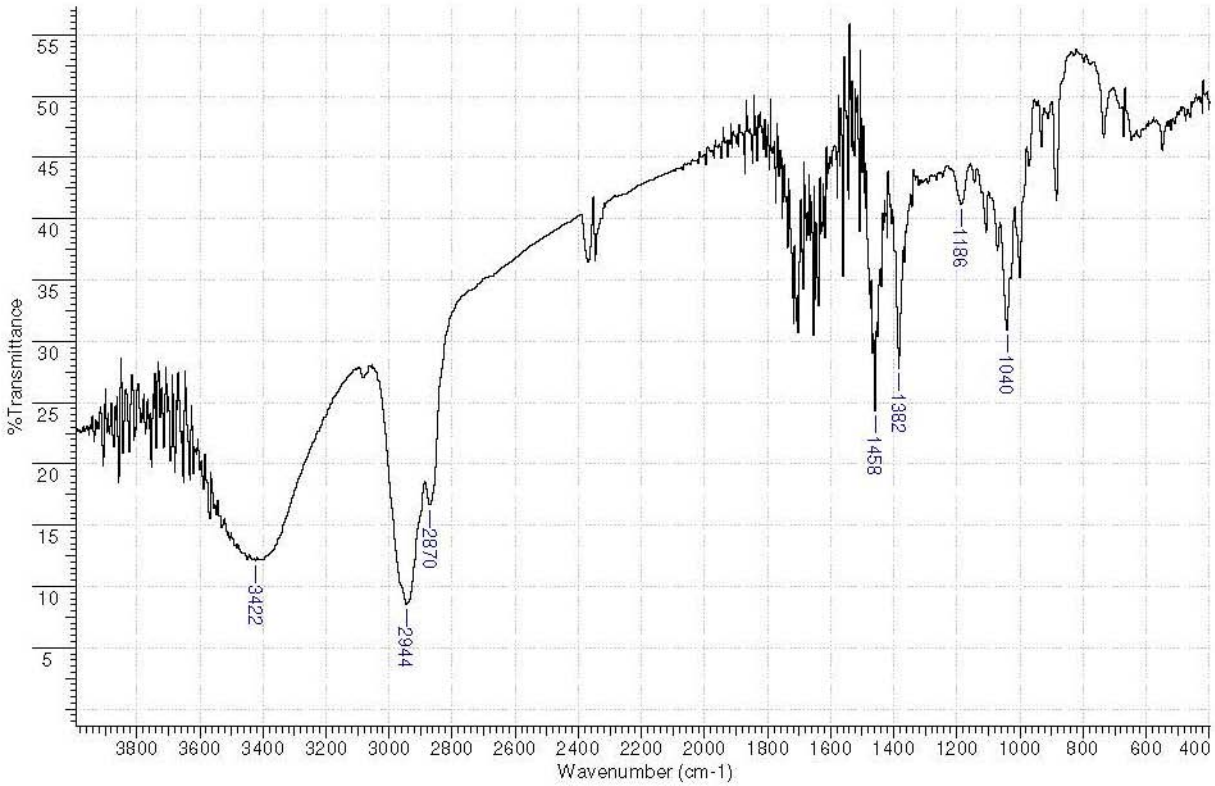

Figure 5S. *IR spectrum of compound **2** (KBr, cm^{-1})*

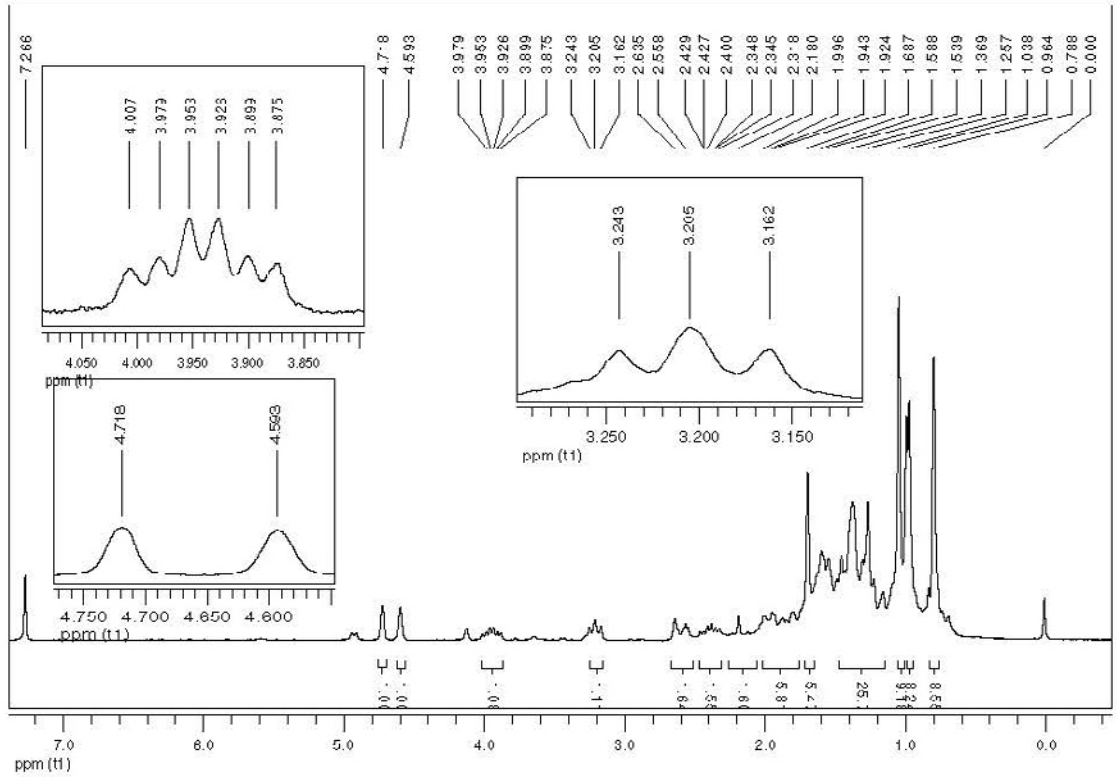

Figure 6S. *[1]H NMR spectrum of compound* ***2*** *(200 MHz, $CDCl_3$)*

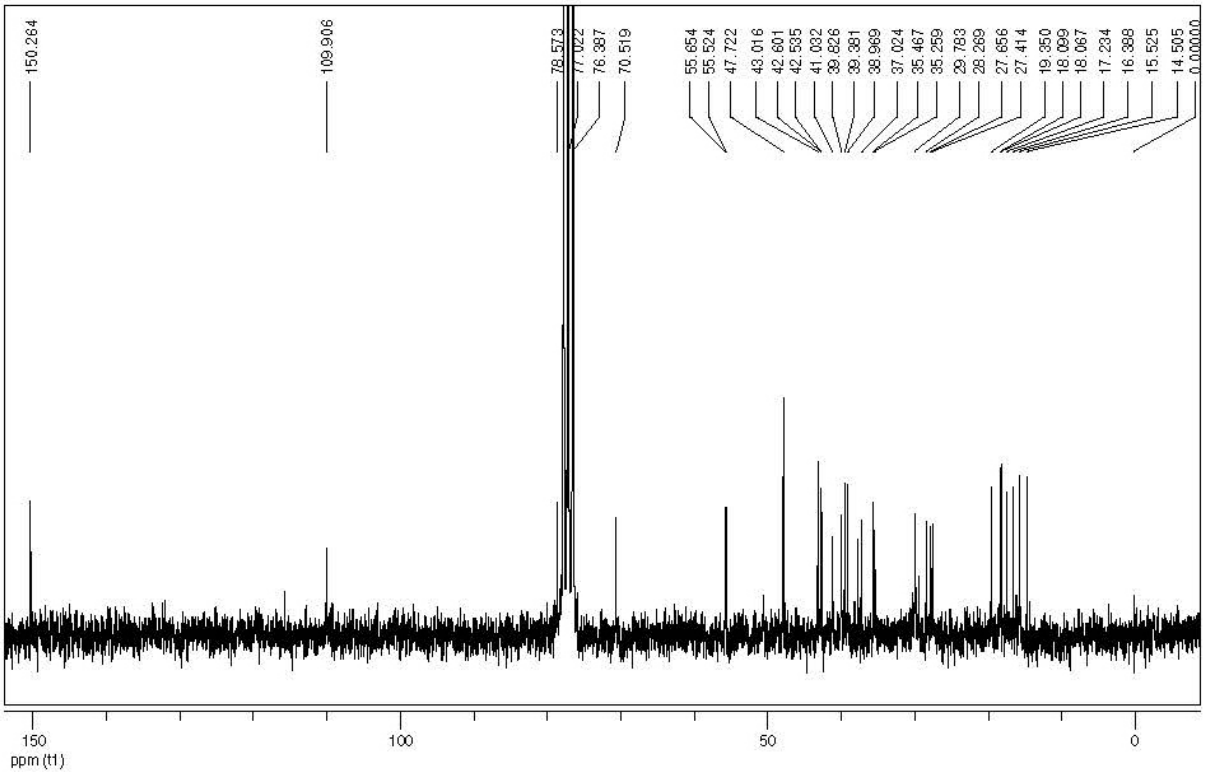

Figure 7S. *[13]C NMR spectrum of compound* ***2*** *(50 MHz, $CDCl_3$)*

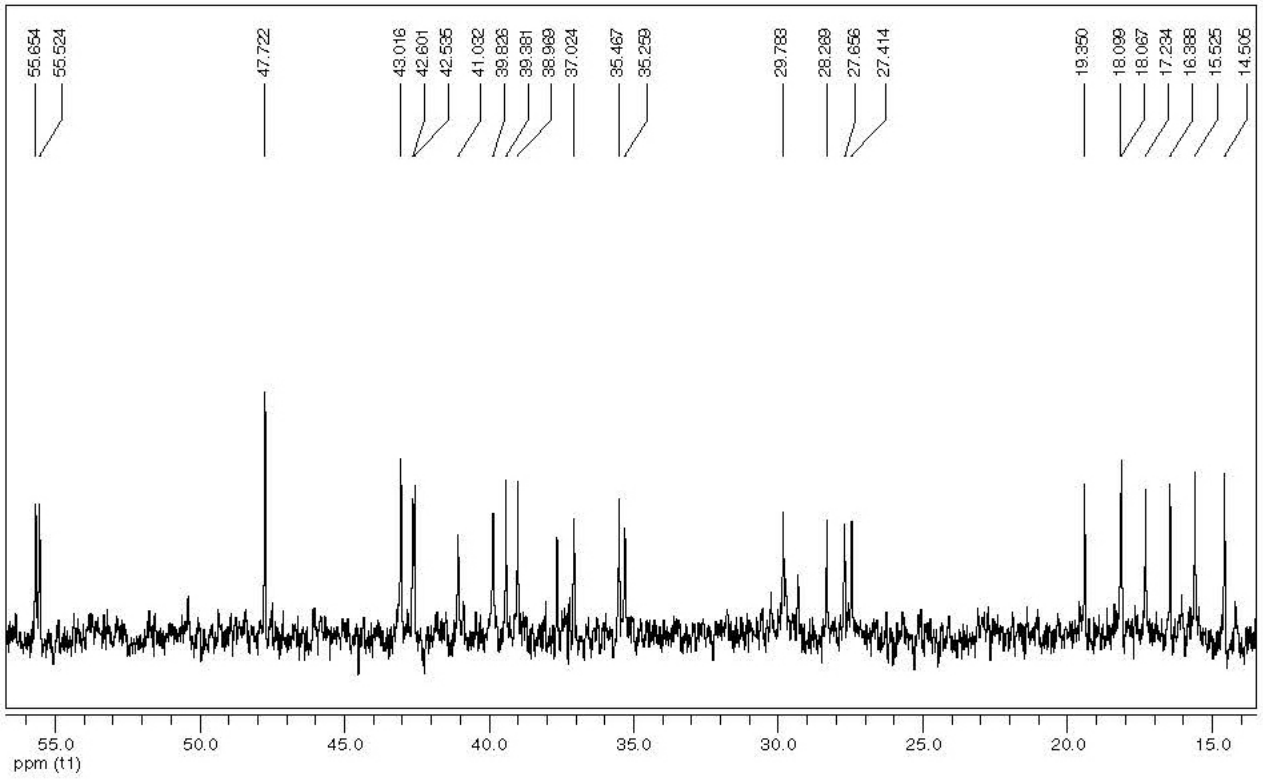

Figure 8S. *^{13}C NMR spectrum of compound **2** (50 MHz, $CDCl_3$)*

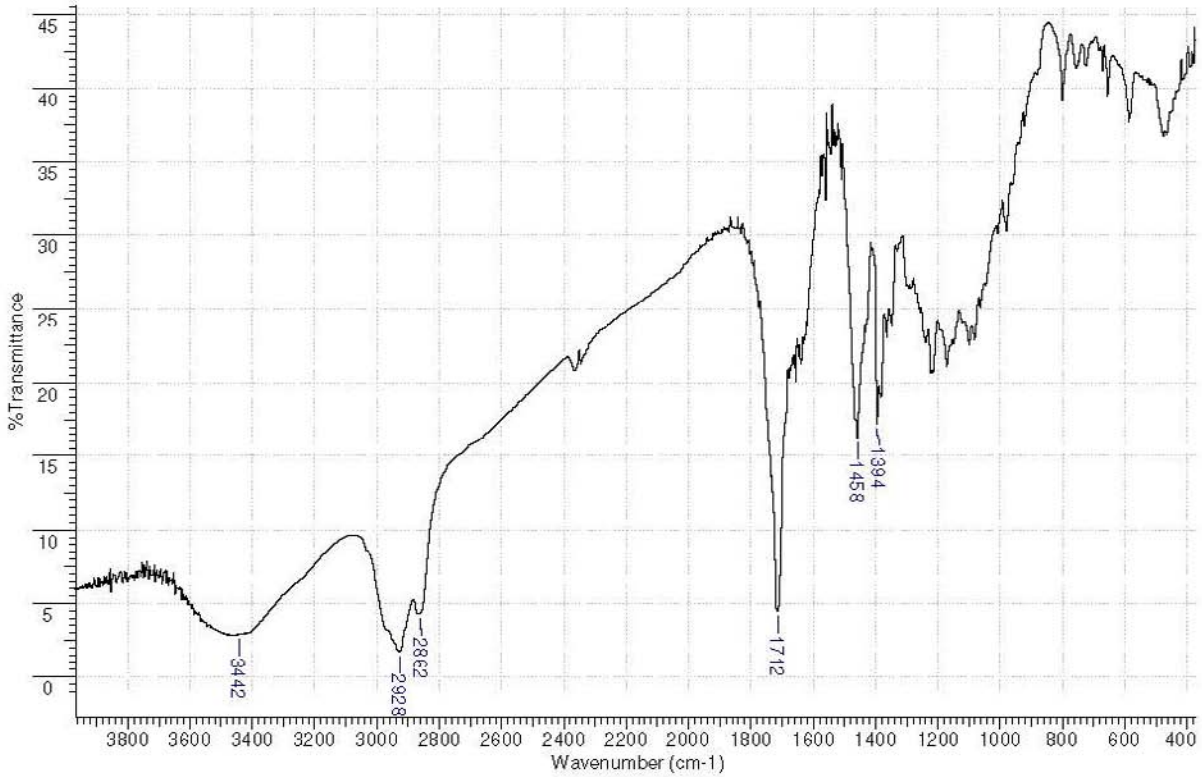

Figure 9S. *IR spectrum of compound **3** (KBr, cm^{-1}). The absorption band at 3442 cm^{-1} was attributed to moisture in the KBr*

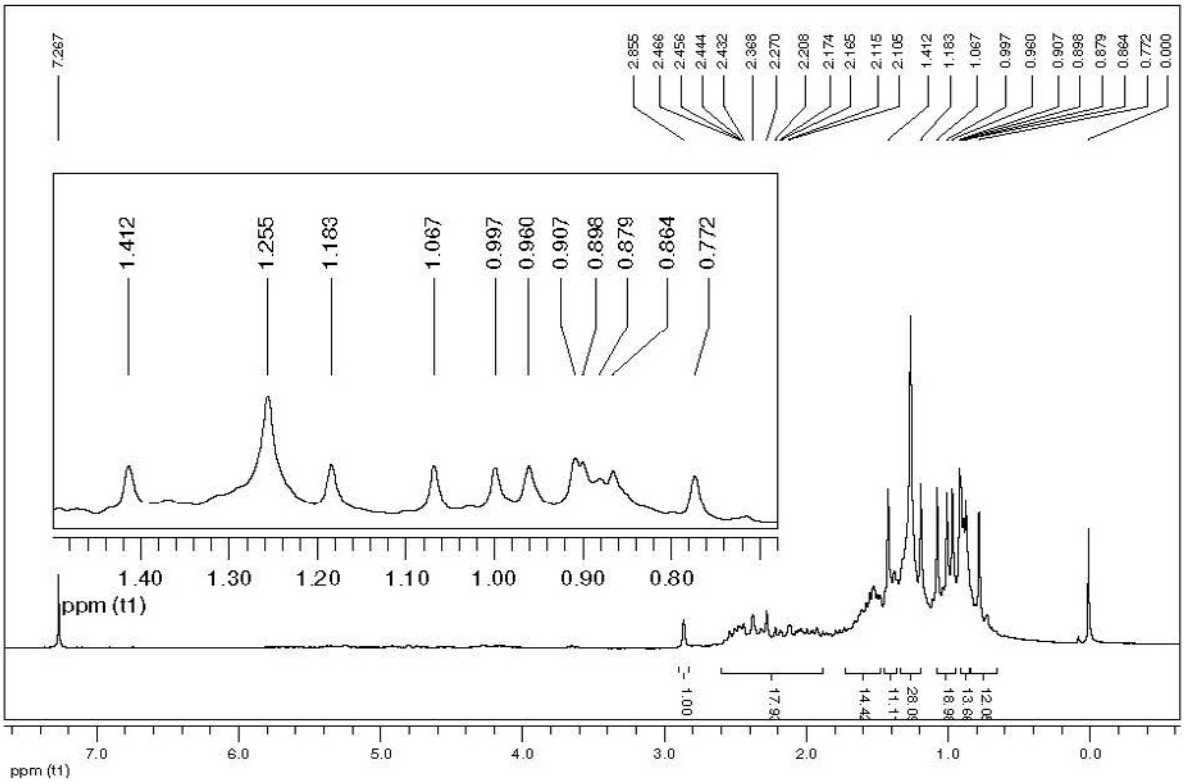

Figure 10S. *[1]H NMR spectrum of compound* ***3*** *(200 MHz, $CDCl_3$)*

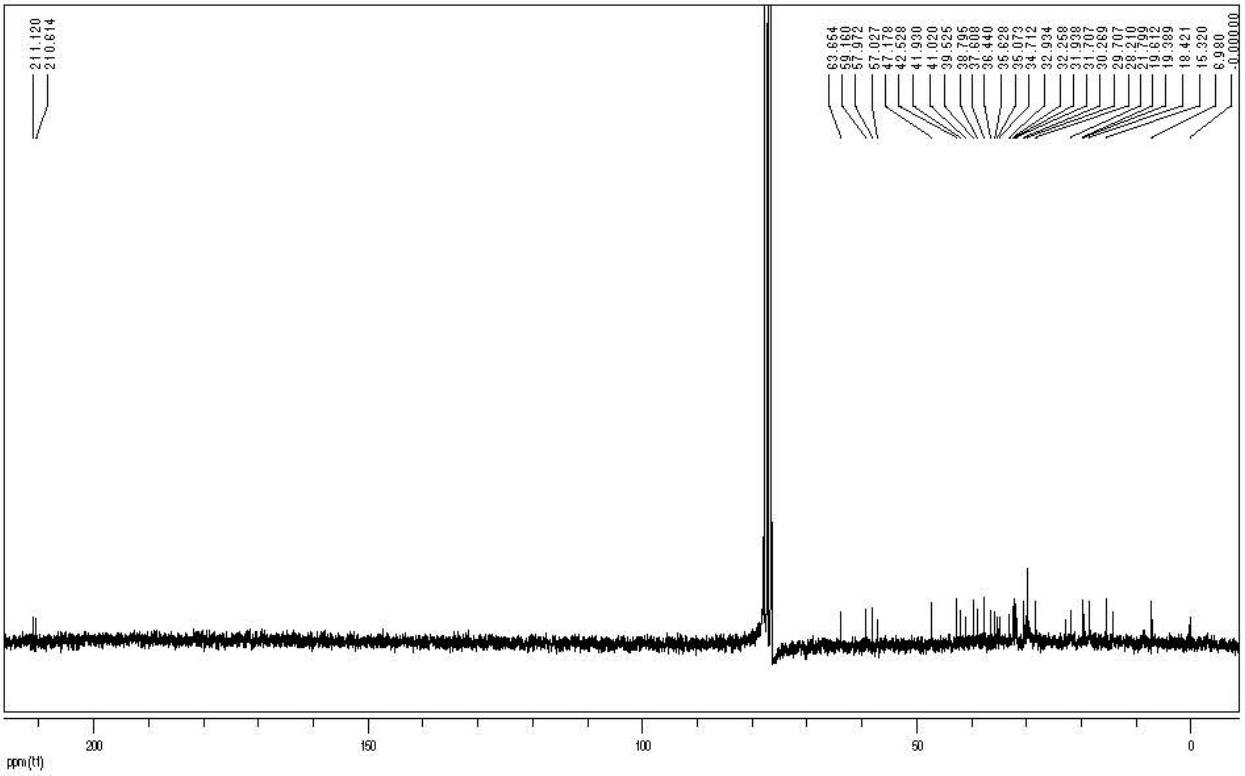

Figure 11S. *[13]C NMR spectrum of compound* ***3*** *(50 MHz, $CDCl_3$)*

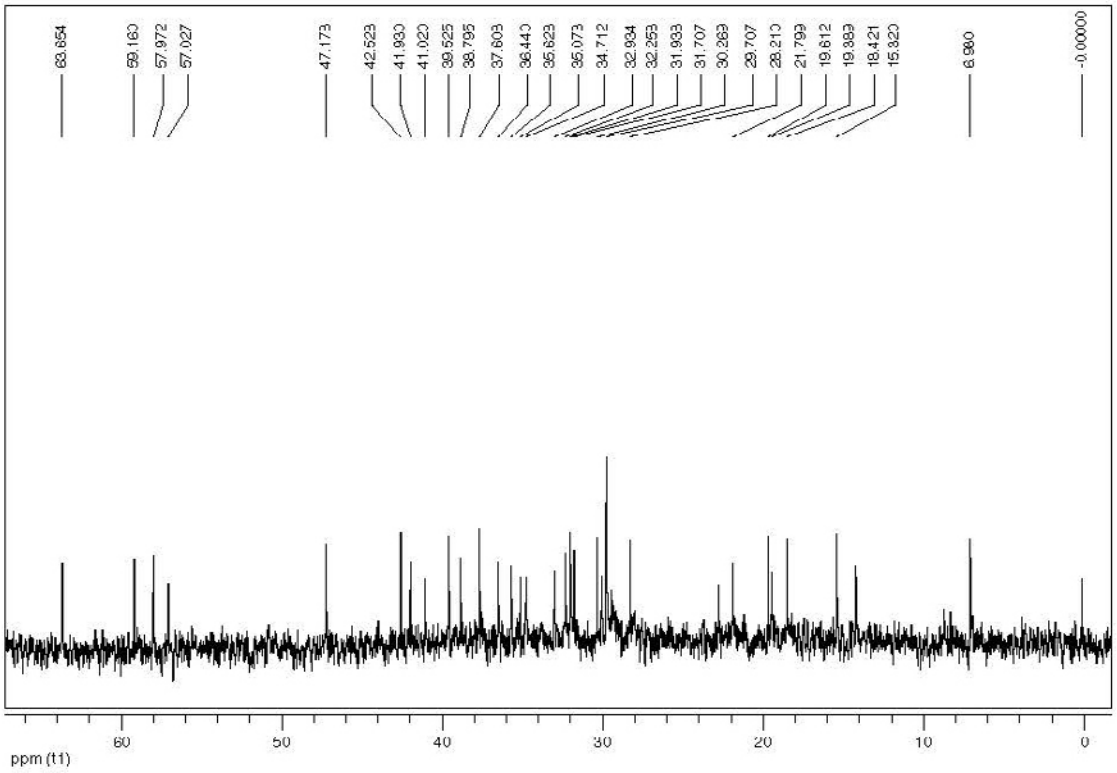

Figure 12S. *^{13}C NMR spectrum of compound **3** (50 MHz, $CDCl_3$)*

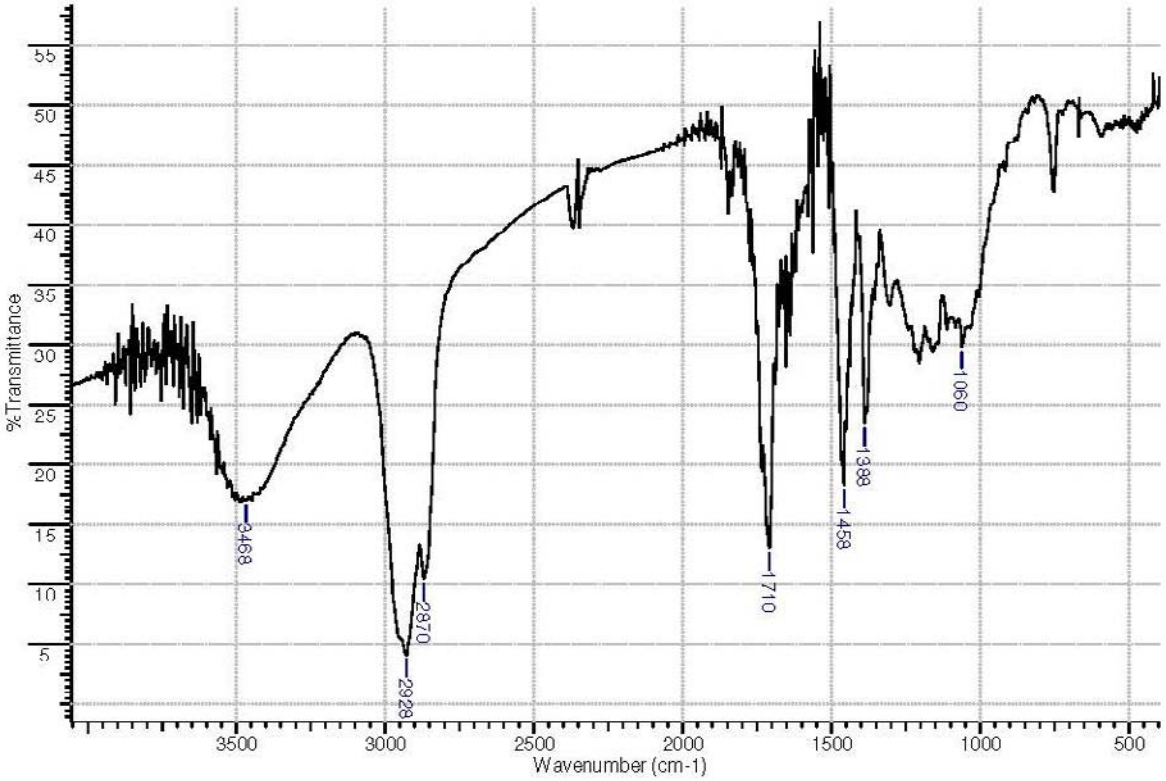

Figure 13S. *IR spectrum of compound **4** (KBr, cm^{-1})*

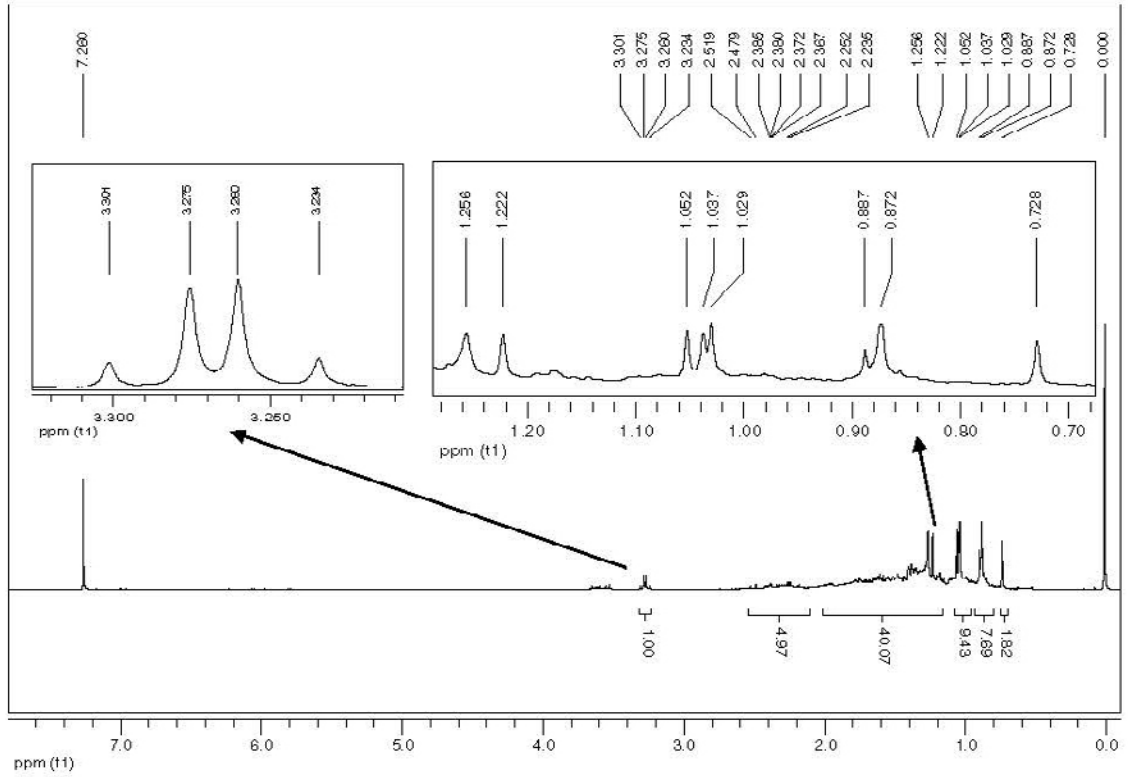

Figure 14S. *[1]H NMR spectrum of compound* ***4*** *(400 MHz, $CDCl_3$)*

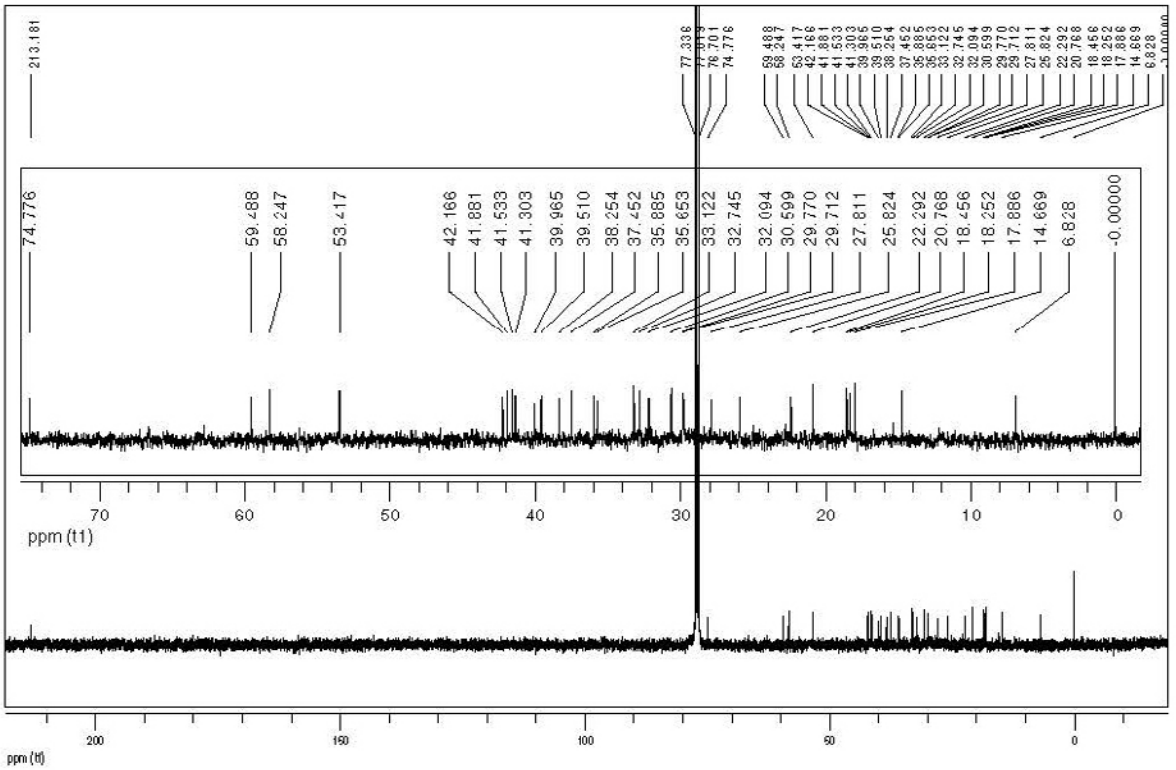

Figure 15S. *[13]C NMR spectrum of compound* ***4*** *(100 MHz, $CDCl_3$)*

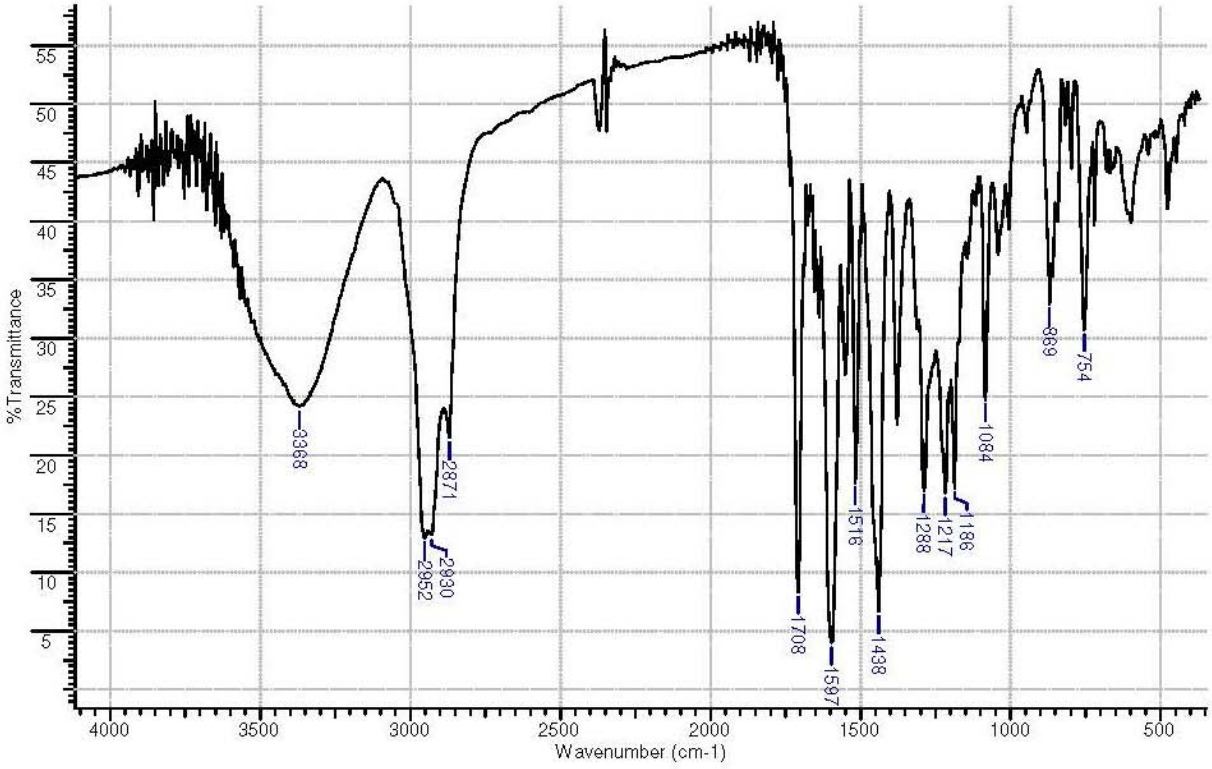

Figure 16S. *IR spectrum of compound* ***5*** *(KBr, cm^{-1})*

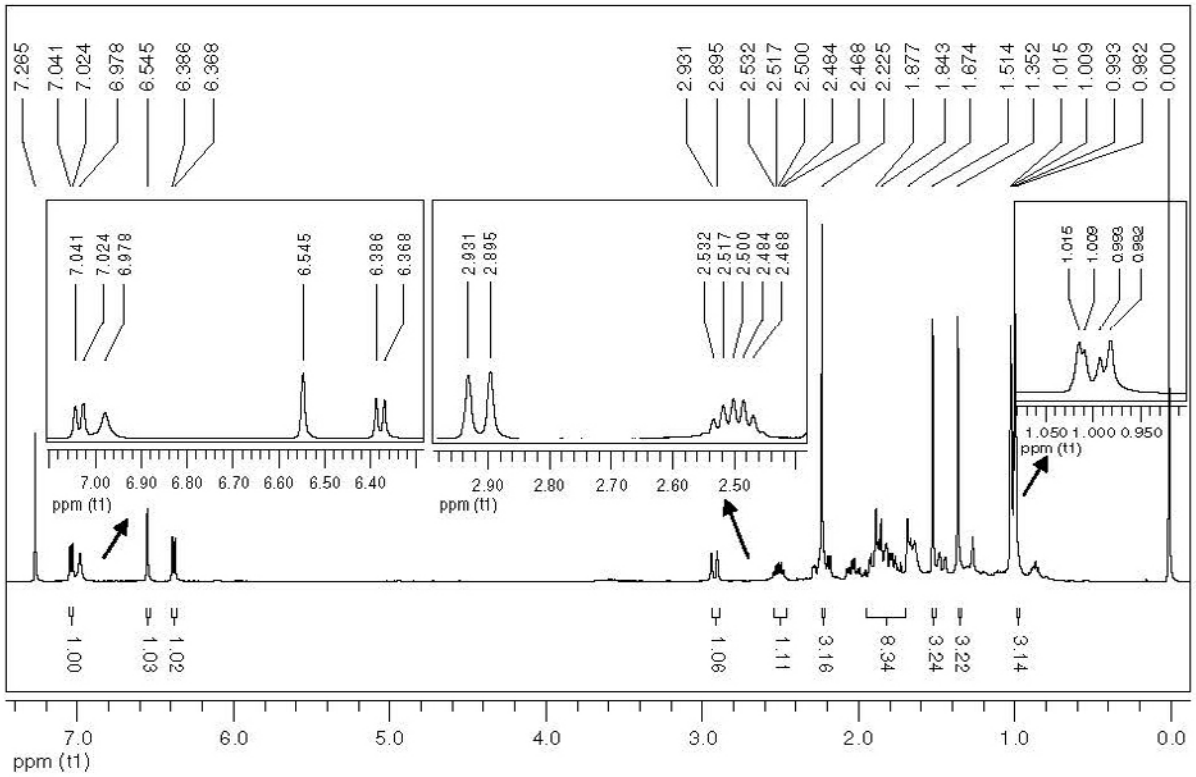

Figure 17S. *^{1}H NMR spectrum of compound* ***5*** *(400 MHz, $CDCl_3$)*

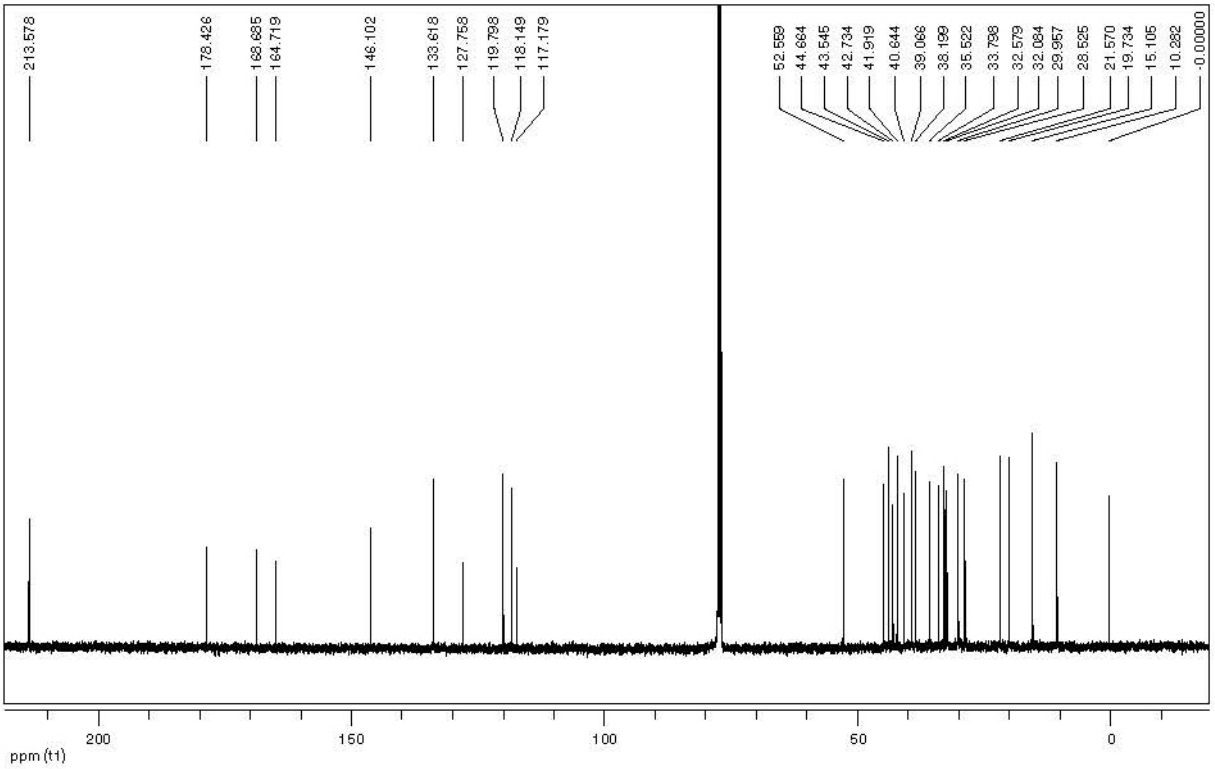

Figure 18S. *^{13}C NMR spectrum of compound* ***5*** *(100 MHz, $CDCl_3$)*

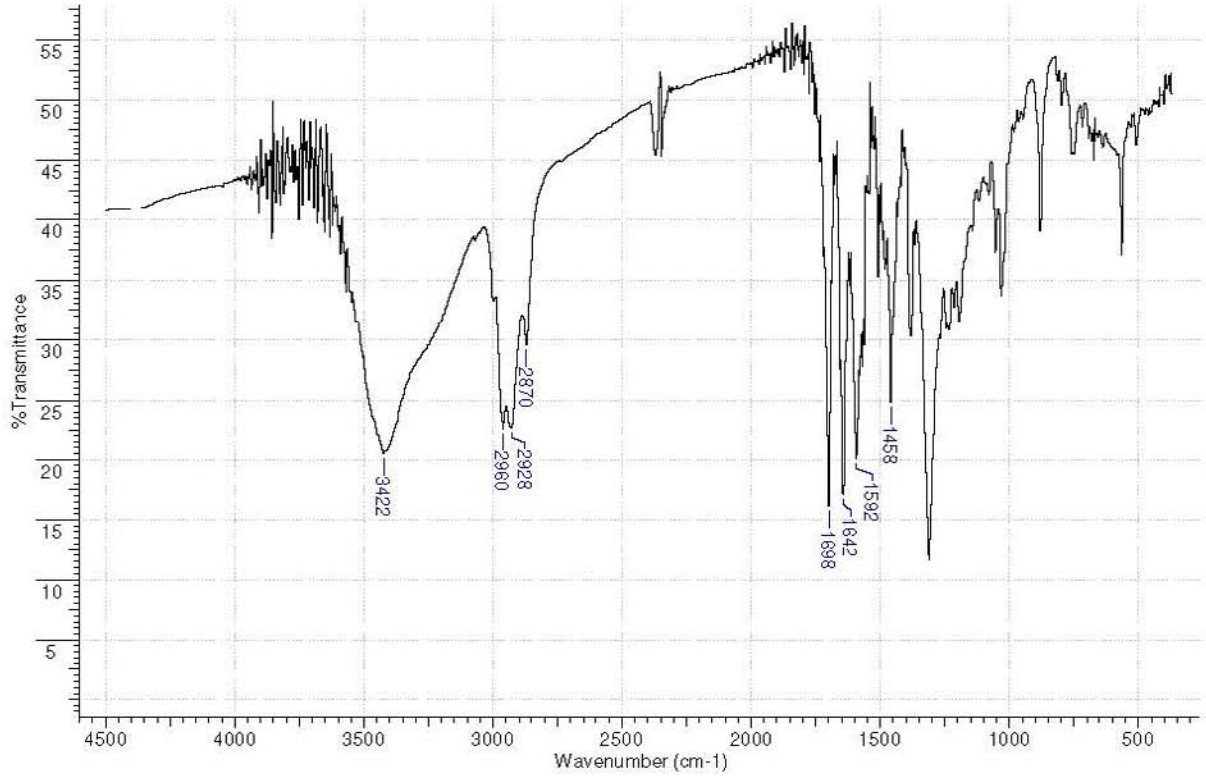

Figure 19S. *IR spectrum of compound* ***6*** *(KBr, cm^{-1})*

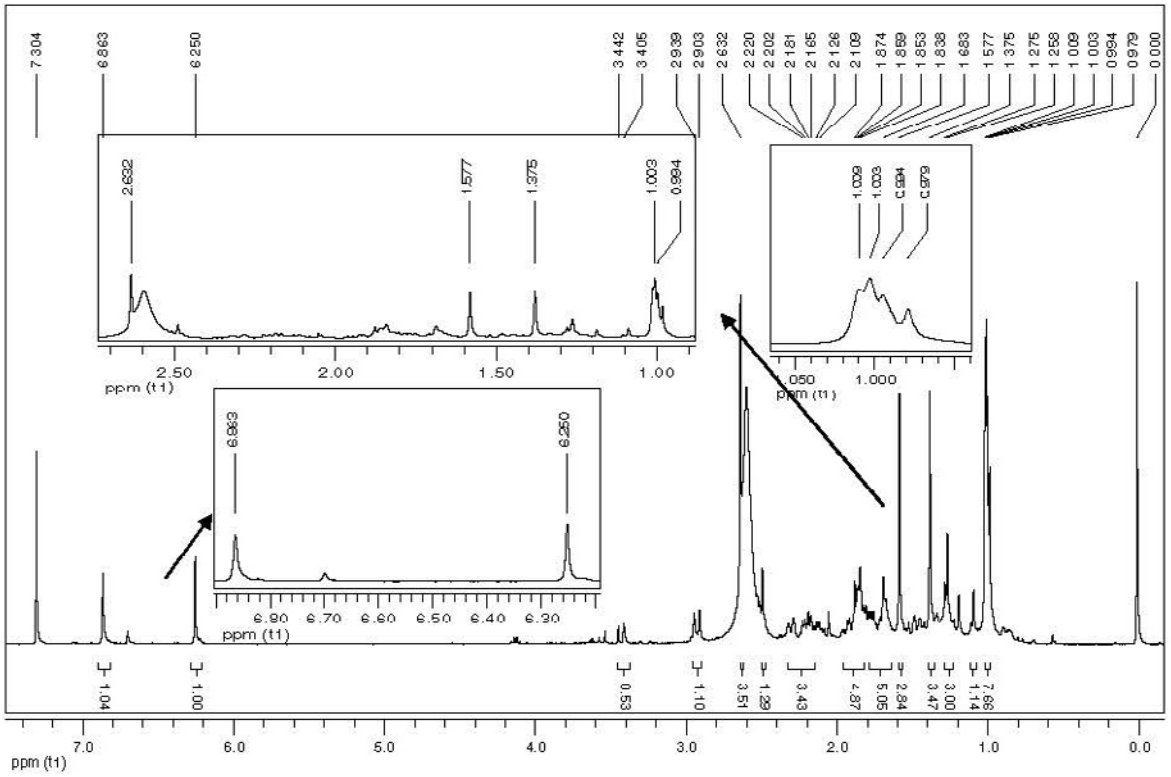

Figure 20S. *^{1}H NMR spectrum of compound **6** (400 MHz, $CDCl_3$ + CD_3OD)*

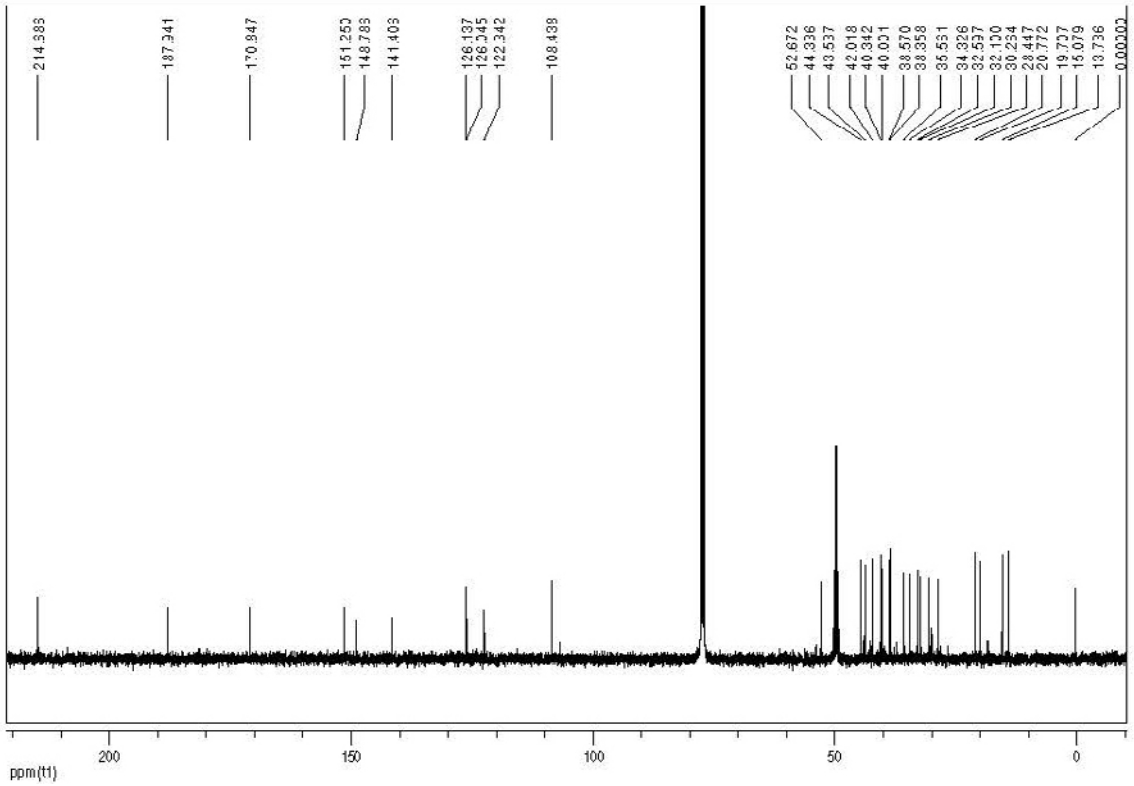

Figure 21S. *^{13}C NMR spectrum of compound **6** (100 MHz, $CDCl_3$ + CD_3OD)*

Table 1S. Comparison of ^{13}C NMR data of compound **1** with literature for 11α-hydroxylup-20(29)-en-3-one

Nº	Type of carbon	δ_C of compound **1**	δ_C ref. 22
1	CH_2	42.09	42.07
2	CH_2	34.23	34.21
3	C=O	218.78	218.84
4	C	47.63	47.63
5	CH	54.78	54.76
6	CH_2	19.66	19.64
7	CH_2	34.27	34.27
8	C	42.63	42.41
9	CH	54.90	54.87
10	C	38.22	38.20
11	CHOH	70.50	70.49
12	CH_2	37.48	37.44
13	CH	37.19	37.17
14	C	42.42	42.16
15	CH_2	27.43	27.41
16	CH_2	35.42	35.40
17	C	43.06	43.05
18	CH	47.65	47.63
19	CH	47.72	47.70
20	C	150.22	150.20
21	CH_2	29.80	29.78
22	CH_2	39.82	39.80
23	CH_3	27.47	27.46
24	CH_3	20.78	20.77
25	CH_3	16.70	16.71
26	CH_3	16.87	16.86
27	CH_3	14.43	14.42
28	CH_3	18.09	18.08
29	$=CH_2$	109.95	109.95
30	CH_3	19.38	19.37

Table 2S. Comparison of ^{13}C NMR data of compound **2** with literature for 3β,11α-hydroxylup-20(29)-en-3-one

Nº	Type of carbon	δ_C of compound **2**	δ_C ref. 21
1	CH_2	39.83	39.00
2	CH_2	27.41	27.50
3	CH	78.57	78.60
4	C	39.38	39.40
5	CH	55.52	55.60
6	CH_2	18.10	18.10
7	CH_2	35.26	35.30
8	C	42.54	41.10
9	CH	55.65	55.70
10	C	38.97	37.70
11	CH	70.52	70.50
12	CH_2	27.66	27.70
13	CH	37.02	37.70
14	C	42.60	42.60
15	CH_2	27.41	27.50
16	CH_2	35.47	35.50
17	C	43.02	43.00
18	CH	47.72	47.70
19	CH	47.72	47.70
20	C	150.26	150.20
21	CH_2	29.78	29.90
22	CH_2	41.03	39.90
23	CH_3	28.27	28.30
24	CH_3	15.53	15.60
25	CH_3	16.39	16.10
26	CH_3	17.23	17.30
27	CH_3	14.51	14.50
28	CH_3	18.07	18.10
29	CH_2	109.91	109.80
30	CH_3	19.35	19.40

Table 3S. Comparison of ^{13}C NMR data of compound **3** with literature for 3,7-dioxo-friedelane

Nº	Type of carbon	δ_C of compound **3**	δ_C ref. 21
1	CH_2	21.80	21.60
2	CH_2	41.02	40.80
3	C=O	211.12	210.60
4	CH	57.97	57.80
5	C	47.18	47.00
6	CH_2	57.03	56.90
7	C=O	210.61	210.20
8	CH	63.65	63.40
9	C	42.53	42.40
10	CH	59.16	59.00
11	CH_2	35.63	35.50
12	CH_2	29.71	29.80
13	C	39.53	39.40
14	C	37.61	37.50
15	CH_2	31.94	31.60
16	CH_2	36.44	36.30
17	C	30.27	30.10
18	CH	41.93	41.80
19	CH_2	35.07	34.90
20	C	28.21	28.00
21	CH_2	32.93	32.80
22	CH_2	38.80	38.60
23	CH_3	6.98	6.80
24	CH_3	15.32	15.10
25	CH_3	18.42	18.20
26	CH_3	19.39	19.20
27	CH_3	19.61	19.40
28	CH_3	32.26	32.10
29	CH_3	31.71	31.80
30	CH_3	34.71	34.60

Table 4S. Comparison of ^{13}C NMR data of compound **4** with literature for 3-oxo-29-hydroxyfriedelane

Nº	Type of carbon	δ_C of compound **4**	δ_C ref. 21
1	CH_2	22.29	22.30
2	CH_2	41.53	41.60
3	C	213.18	212.20
4	CH	58.25	58.30
5	C	42.17	42.20
6	CH_2	41.30	41.40
7	CH_2	18.25	18.30
8	CH	53.42	53.50
9	C	37.45	37.50
10	CH	59.49	59.60
11	CH_2	35.65	35.70
12	CH_2	29.71	29.80
13	C	39.97	40.00
14	CH	38.25	38.30
15	CH_2	32.75	32.80
16	CH_2	35.89	36.00
17	C	29.77	29.80
18	CH	41.88	42.00
19	CH_2	30.60	30.60
20	C	33.12	33.20
21	CH_2	27.81	27.90
22	CH_2	39.51	39.60
23	CH_3	6.83	6.80
24	CH_3	14.67	14.70
25	CH_3	17.89	17.90
26	CH_3	18.46	18.40
27	CH_3	20.77	20.80
28	CH_3	32.09	32.10
29	CH_2	74.78	74.80
30	CH_3	25.82	25.90

Table 5S. Comparison of ^{13}C NMR data of compound **5** with literature for tingenone

Nº	Type of carbon	δ_C of compound **5**	δ_C ref. 23
1	CH	119.80	119.80
2	C	178.43	178.40
3	C	146.10	146.00
4	C	117.18	117.10
5	C	127.76	127.70
6	CH	133.62	133.60
7	CH	118.15	118.10
8	C	168.69	168.70
9	C	42.73	42.70
10	C	164.72	164.70
11	CH_2	33.80	33.80
12	CH_2	29.96	29.90
13	C	40.64	40.60
14	C	44.66	44.60
15	CH_2	28.53	28.50
16	CH_2	35.52	35.50
17	C	38.20	38.20
18	CH	43.55	43.50
19	CH_2	32.08	32.00
20	CH	41.92	41.80
21	C	213.58	213.60
22	CH_2	52.56	52.50
23	CH_3	10.28	10.20
25	CH_3	39.07	39.00
26	CH_3	21.57	21.50
27	CH_3	19.73	19.70
28	CH_3	32.58	32.50
30	CH_3	15.11	15.10

Table 6S. Comparison of ^{13}C NMR data of compound **6** with literature for 6-oxo-tingenol

Nº	Type of carbon	δ_C of compound **6**	δ_C ref. 24
1	CH	108.44	108.19
2	C	148.79	148.87
3	C	141.41	141.42
4	C	126.14	125.87
5	C	122.34	121.71
6	C=O	187.94	187.90
7	CH	126.05	125.53
8	C	170.85	171.02
9	C	40.34	40.07
10	C	151.25	150.99
11	CH_2	35.56	35.23
12	CH_2	30.26	29.93
13	C	40.00	39.75
14	C	44.34	44.02
15	CH_2	28.45	28.14
16	CH_2	32.10	31.81
17	C	38.36	38.18
18	CH	43.54	43.23
19	CH_2	34.33	33.99
20	CH	42.02	41.74
21	C=O	214.69	215.08
22	CH_2	52.67	52.35
23	CH_3	13.74	13.23
25	CH_3	38.57	38.09
26	CH_3	20.77	20.40
27	CH_3	19.71	19.30
28	CH_3	32.60	32.12
30	CH_3	15.08	14.55