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ANTIMICROBIAL ACTIVITIES AND THE FIRST ISOLATION OF 4-NITROBENZOIC ACID TETRAHYDROFURAN-2-YL-METHYLESTER AND 4-HYDROXY-5-METHYLFURAN-3-ONE FROM TERRESTRIAL *STREPTOMYCES* SP.

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ABSTRACT

In our investigation of the crude extracts from the terrestrial derived Streptomycete isolate sp. *Wo* 990, two compounds namely, 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester (**1**) and 4-hydroxy-5-methylfuran-3-one (**5**) were obtained for the first time from microorganisms. In addition, 2-phenylacetamide, 5-methyl-1H-pyrimidine-2,4-dione, 2-amino-3-phenyl-propionic acid and 2-(6-amino-purin-9-yl)-5-methyl-sulfanylmethyl-tetrahydrofuran-4-diol were isolated. The structures of the new compounds **1** and **5** were elucidated on the basis of spectroscopic analysis (1D and 2D), mass spectra and comparison with the literature data.

KEYWORDS: **Terrestrial streptomycetes, Nitrobenzoic acid derivative, Furanone, Natural products.**

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INTRODUCTION

Natural products are major sources of drugs for the treatment of various health disorders[1,2]. The use of natural products as a remedy has been known since 4000-5000 B.C. Nowadays, huge numbers of allopathic medicines also contain natural products based ingredients that are used for their preparation. Thus, natural products have played a significant role in maintaining human health and improving the quality of human life for thousands of years, which furnished us with valuable components of seasoning beverages, cosmetics and dyes. The search for new pharmacologically active agents obtained from the plants has led to the discovery of many clinically useful drugs that play a major role in treatment of human disease[3]. Natural products such as polyphenols, have properties including antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antiviral, and anti-microbial effects that might potentially be beneficial in preventing diseases and protecting the stability of the genome[2-5]. Microorganisms have recently be targeted as a very interested source of novel biologically active compounds. Nitro compounds are rare in nature and the most well-known is the antibiotic chloramphenicol, which has been isolated from bacteria[6-10]. In our screening program of Streptomycetes for new novel compounds, we isolated a new natural 4-nitrobenzoic acid tetrahydrofuran-2-yl-methylester **(1),** and 4-hydroxy-5-methylfuran-3-one (**5**). Both compounds (**1**) and (**5**) are synthetically well known[11-13].

MATERIALS AND METHODS

General

NMR spectra were measured on Varian Unity 300 and Varian Inova 600 spectrometers. Electron spray ionization mass spectrometry (ESI HRMS): Finnigan LCQ ion trap mass spectrometer coupled with a Flux Instruments (Basel, Switzerland) quaternary pump Rheos 4000 and a HP 1100 HPLC (nucleosil column EC 125/2, 100-5, C 18) with a Diode Array Detector (Finnigan Surveyor LC System). High resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV), Thermo Electron Corp., Bremen, Germany; with perfluorkerosene as reference substance for EI HRMS. UV-VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrometer. Flash chromatography was carried out on silica gel (230-400 mesh). R_f-values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Microorganism Material

The actinomycetes strains were obtained from different localities of BeniSuef governorate, Egypt. Both a traditional morphological assessment and 16S rDNA sequence analysis were performed to characterize it as *Streptomyces* sp.

Fermentation and Isolation

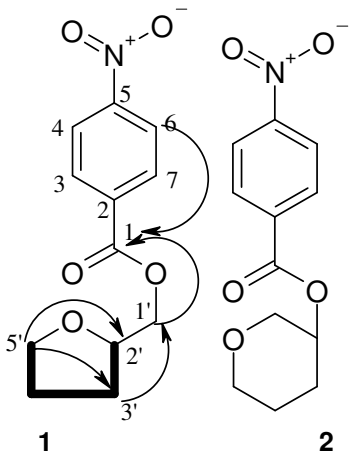
The terrestrial isolate *sp.Wo*990 was pre-cultivated on M_2 agar plates at 28 °C for 3 days. With pieces of a well-grown agar subculture, a 25 L cultivated in a 25-liter scale on M_2 medium at 28°C for 5 days on a linear shaker (110 rpm). The culture broth was mixed with *ca.* 1.5 kg Celite and filtered under pressure. The water phase was extracted with a XAD-16, the resin washed with 20 L water and eluted with 15 L methanol, while the mycelium was extracted firstly with ethyl acetate (3 times) and then acetone (1 time). Both extracts were combined in based on TLC, evaporated to dryness affording 4 g yellowish-brown crude extract. Chromatography of the crude extract (4 g) obtained, was performed using silica gel with a MeOH/CH_2Cl_2 gradient delivering four fractions F1-F4 as in (fig. 1). Fraction 1 and 2 were identified as fat acids based on TLC and spraying with anisaldehyde/H2SO4 reagent. Purification of fraction F3 on Sephadex LH-20 (MeOH) and *RP*18 (30% MeOH) delivered 4-nitrobenzoic acid tetrahydro-furan-2-yl-methylester (**1**)**.** (20 mg) and 4-hydroxy-5-methylfuran-3-one (**5**) (50 mg). Treatment of fraction 4 with Sephadex LH-20 (MeOH) and *RP*18 afforded four pure compounds 2-phenylacetamide, (5 mg) 5-methyl-1H-pyrimidine-2,4-dione, (4 mg) 2-amino-3-phenylpropionic acid (6 mg) and 2-(6-amino-purin-9-yl)-5-methylsulfanyl-methyl-tetrahydrofuran-,4-diol (30 mg). The structure of the known compounds easily identified by sub-structure searches in AntiBase data [14] (see fig. S1).

4-Nitrobenzoic acid tetrahydrofuran-2-yl-methyl-ester (1)

Colourless solid, green coloration with anisalde-hyde/sulfuric acid spraying reagent.
[α] D: 1.2 (c 1.00, MeOH).
Rf: 0.75 (CH_2Cl_2/1% MeOH). Melting point 45-8 ^{0}C
UV/VIS: λmax (0.1 mg/ml MeOH): (MeOH) 320, 380
^{1}H NMR (300 MHz, CD3OD): see Table 1
^{13}C NMR (125 MHz, CD3OD): see Table 1
(-)-ESI MS m/z (%) 520 ([2M+NH4]+, 15), 252 ([M+H]+, 20), 269 (M + NH4+, 100).
ESIHR MS: m/z $[M+H]^+$calcd: for $C_{12}H_{13}NO_5$: 252.08679; found 252.08665.
m/z $[M+Na]^+$ calcd for $C_{12}H_{13}NO_5Na$: 274.06872, found 274.6859

4-Hydroxy-5-methylfuran-3-one (5)

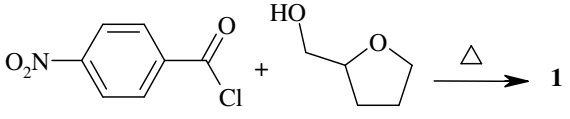
Coulourless solid, brownish green coloration with anisaldehyde/sulfuric acid spraying reagent.
R_f = 0.64(CH_2Cl_2/ 2% MeOH).
^{1}H NMR (300 MHz, CD_3OD): see Table 1
^{13}C NMR (125 MHz, CD_3OD): see Table 1
EI MS (70 eV): m/z (%) 114 ($[M]^+$, 75), Calcd. for $C_5H_6O_3$



1 **2**

Figure 1

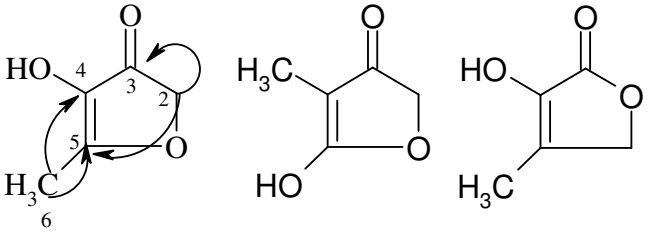
Selected ^{1}H-^{1}H COSY (—) and HMBC (→) correlations of compound (1)



3 **4**

Figure 2

Synthesis of compound 1



5 **6** **7**

Figure 3

Selected HMBC (→) correlations of 4-hydroxy-5-methylfuran-3-one (5)

2.2. Antimicrobial activities

The antimicrobial activities of the crude extract and compound **1** were tested against different test organisms by broth microdilution method [15].

RESULTS AND DISCUSSION

Structure elucidation

Cultivation of the *Stryptomyces* spp. *Wo* 990, in M_2 medium (M2 medium: 10 g malt ¬extract, 4 g yeast extract and 4 g glucose in 1 l of tap water was set to pH 7.8 with 2 N NaOH and sterilized for 30 min at 121 °C) at 28°C in a 25-liter shaker culture scale delivered a yellowish-brown culture broth which was worked up in the usual manner. TLC-directed work-up of the resulting crude extract was separated by a sequence of chromatographic steps (fig. S1). Compound 1 was isolated as an UV absorbing (254 nm), blue fluorescence (366 nm) colourless solid which turned to greenish-blue on spraying with anis-aldehyde/sulphuric acid reagent. The ESI MS of compound 1 indicated a molecular weight of *m/z* 251, and ESI HRMS confirmed the molecular formula as $C_{12}H_{13}NO_5$ entailing seven double bond equivalences. The UV spectra (MeOH) of 1 displayed two strong bands at λ_{max} = 320 and 380 nm. ^{1}Hand ^{13}CNMR data were listed inTable1.The ^{1}H NMR spectrum in CD_3OD exhibited a doublets of two *ortho*-coupled protons each of 1H at δ 8.32 (d, *J* = 8.98 *Hz*) and 8.24 (d, *J* = 8.98 *Hz*) in the aromatic region, indicating the presence of 1,4-disubistituted benzene ring. In the aliphatic region, it showed multeplets of three oxygenated groups one of 2H for an oxy-methylene group at δ 4.39; another oxymethylene group at δ 3.87 and one CH group at δ 4.27. Additionally, a multiplet of CH_2 at δ 1.93 and 1.76, 2.09. ^{13}C NMR and HMQC spectrum of 1 indicated the presence of 12 carbon signals, of which a carbonyl group at δ 165.9, six sp^2 carbons of the benzene ring (two carbons which were down field C-5 at δ 152.1 which, was directly attached to nitro group and C-2 at δ136.8), attached to carbonyl group, as well as four methine carbons C-3, C-4, C-6 and C-7 at δ124.6, 131.9, 131.9 and 124.6 respectively. On the other hand, the sp^3 region displayed signals of oxy-carbons at δ 68.5, of C-1', 77.9 of C-2' and 69.5 of C-5'. From the reveled spectroscopic data, there are two structure possibilities for 1 and 2. Subjecting the compound for HMBC measurement, a ^{3}J correlation from the methyl group (δ 4.39) to carbonyl carbon (165.9) was observed. On the other hand, compound 1 was

confirmed by synthesis[16] according to the reaction of 4-nitrobenzoylchloride 3 with furfuryl alcohol 4 as in (fig. 2). Comparison of all data of the synthesized structure with the naturally isolated one gave the same data ^{1}HNMR, melting point, R_f value and finally the same color reaction with anisaldehyde /sulfuric acid spraying reagent so, compound 2 was excluded.

Table 1
^{1}H and ^{13}C NMR assignments of compounds 1 and 5 (J in Hz)

No.	Compound 1		No.	Compound 5	
	δ_C [a]	δ_H [b]		δ_C [a]	δ_H
1	165.9	-	2	73.0	4.49 (s)
2	136.8	-	3	196.2	-
3,7	124.6	8.24 (d, 8.9)	4	135.2	-
4,6	131.9	8.32 (d, 8.9)	5	176.2	-
5	152.1	-	6	13.6	2.42 (s)
1'	68.5	4.31, 439 (m)			
2'	77.9	4.27 (m)			
3'	28.8	1.76, 2.09 (m)			
4'	26.7	1.93 (m)			
5'	69.5	3.87 (m)			

The second compound 5 was obtained as a colorless solid and shows the brownish green color with anisaldehyde/sulfuric acid spraying reagent. The molecular weight was deduced by EI MS as m/z 114, and HREI MS resulted in the molecular formula $C_5H_6O_3$. The ^{1}H and ^{13}C NMR spectrum of 5 are listed in Table 1. The ^{1}H NMR spectrum in CD_3OD showed singlet protons at δ 4.49 of the oxygenated methylene group and other singlet protons at 2.42 of one methyl group.The ^{13}C NMR and HMQC spectra of 5 revealed the presence of 5 carbon signals, of which, a carbonyl group at δ 196.2, two $sp^2\alpha$, β carbons in down field region C-4 at δ 135.2 and C-5 at δ 176.2 In addition, two signals in the sp^3 region for methyl group at δ 13.6 and oxygenated methylene group at δ 73.0 were present. From the above spectroscopic data, there are three structure possibilities 5, 6 and 7. According to the HMBC measurement, a strong correlation from the methyl group (δ 2.42) to the carbon at (135.2) was observed but very low correlation of methyl group to carbon (176.2) was observed. Thus, compound 6 was excluded. On the other hand, according to the high value of carbonyl carbons (196.2), indicated ketonic carbon and not ester carbon. Then, compound 7 was also excluded.

Antimicrobial activities

The crude extract of the strain *Streptomyces* sp. isolate *Wo* 990 exhibited biological activity against the bacteria *Bacillus subtilis*, *Escherichia coli*, and *Streptomyces viridochromogenes*, the fungi *Candida albicans* and *Mucormiehei; against algae, Chorella vulgaris and Chorellasubspicatus and* Scenedesmus*subspicatus* responsible for the antifungal and also gave 90% activity against *Brine schrimp*but the pure compound **1** gave 54 % activity. See Table **2.**

Table 2
Antimicrobial activities of crude extract and compound 1

Test organisms	Inhibition zone in Φ [mm] of Crude extract	Inhibition zone in Φ [mm] of Compd. 1
Bacillus subtilis	18	0
Staphyloccus aureus	22	0
St.viridochromogenes (Tü)	20	0
Escherichia coli	22	0
Candida albicans	20	0
Mucor miehei (Tü 284)	14	0
Chlorella vulgaris	0	23
Chlorella sorokiniana	11	34
Scenedesmussubspicatus	0	30
Brine schrimp	90 %	54 %

CONCLUSION

Overall, the present investigation reveals the first isolation of two bioactive natural from terrestrial Streptomyces, along with four previously isolated natural compounds. The crude extract and compound one were shown to possess good biological activities as antimicrobial agents.

ACKNOWLEDGMENT

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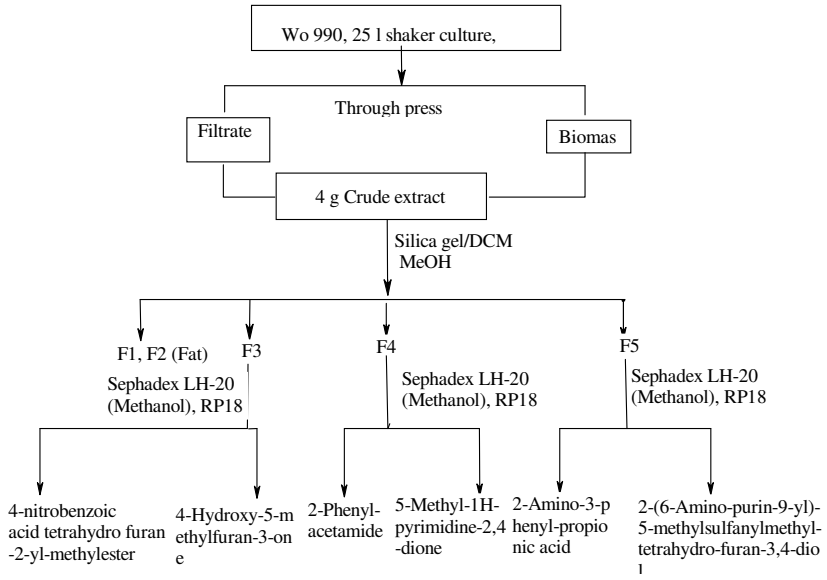
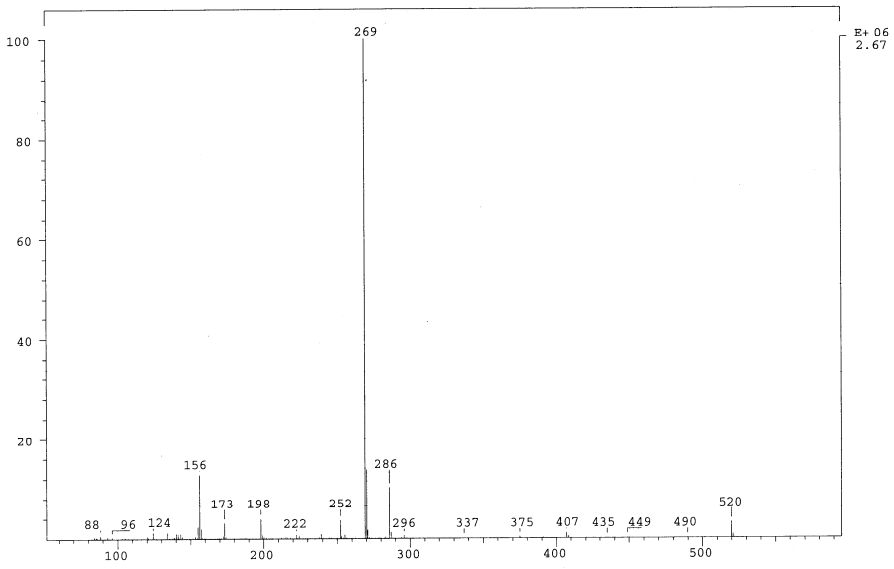
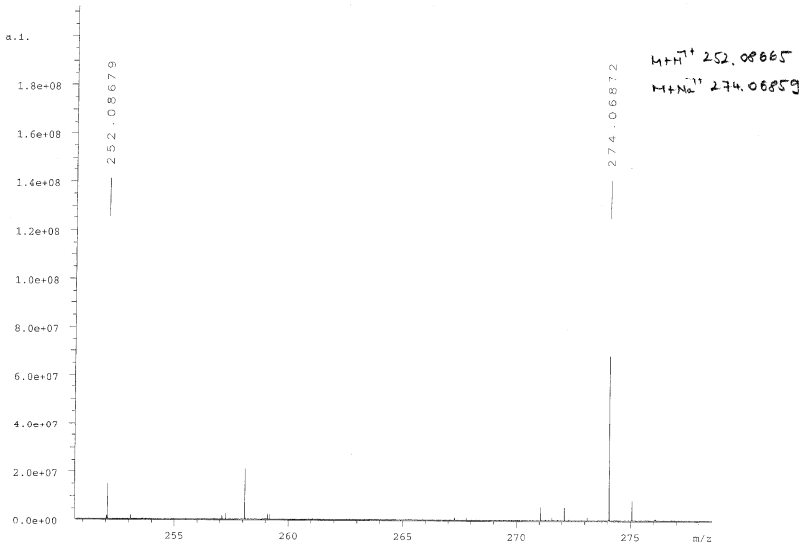
SUPPORTING INFORMATION



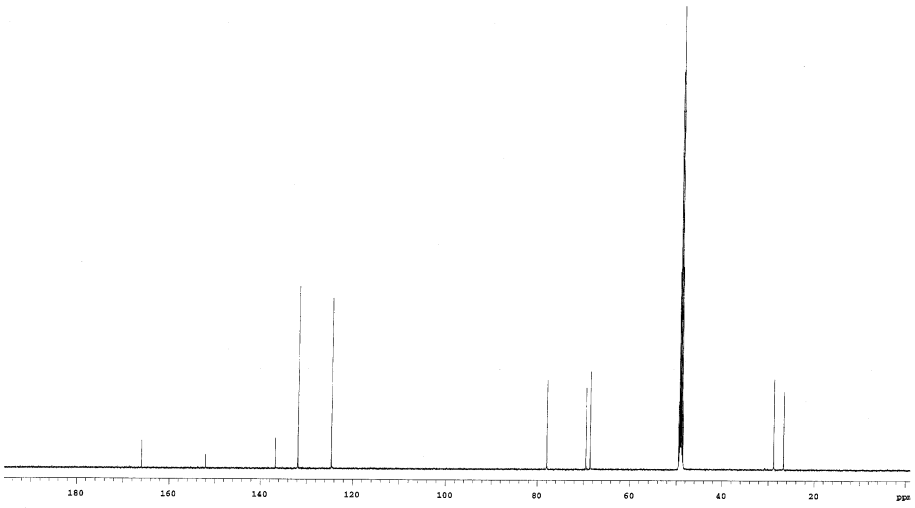
S1
Working up scheme of Streptomyces sp. WO 990



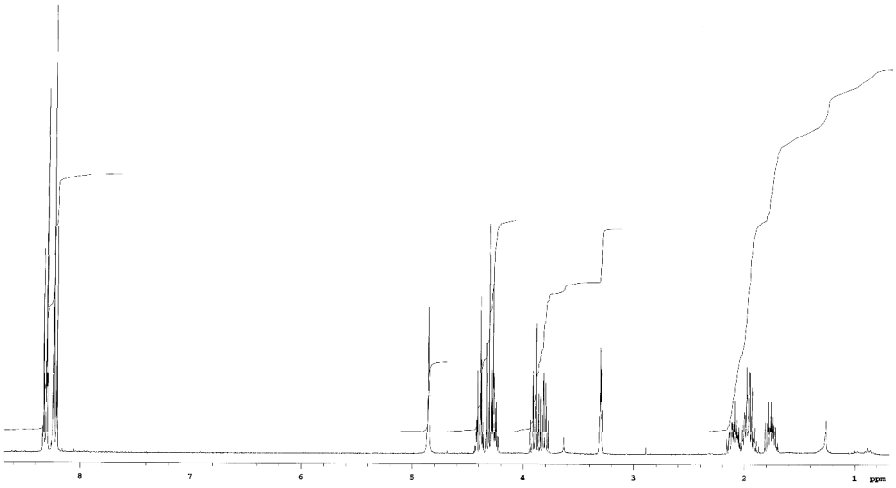
S2
(-)-ESI MS Spectrum of Compound 1



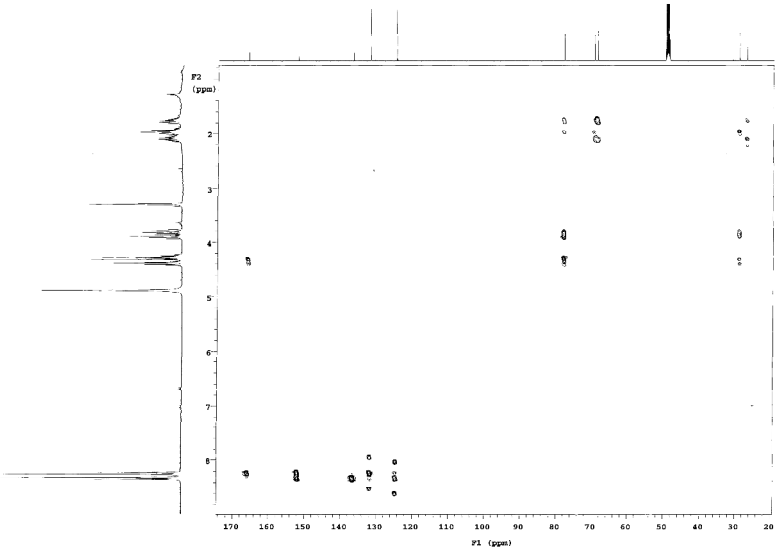
S3
HRESI-MS Spectrum of Compound 1



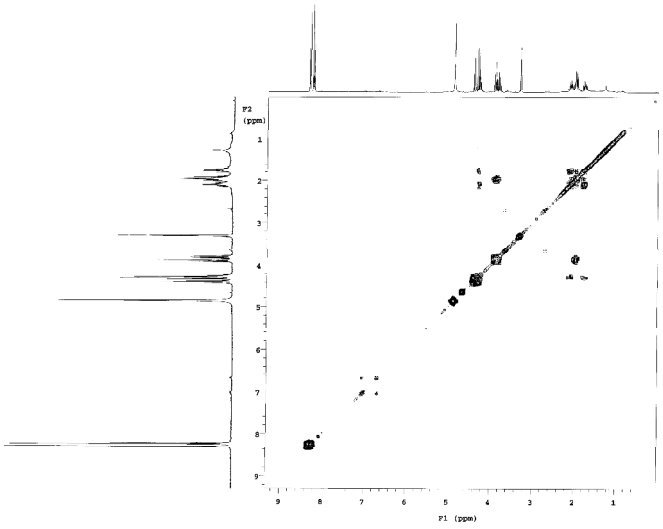
S4
^{13}C-NMR Spectrum of Compound 1



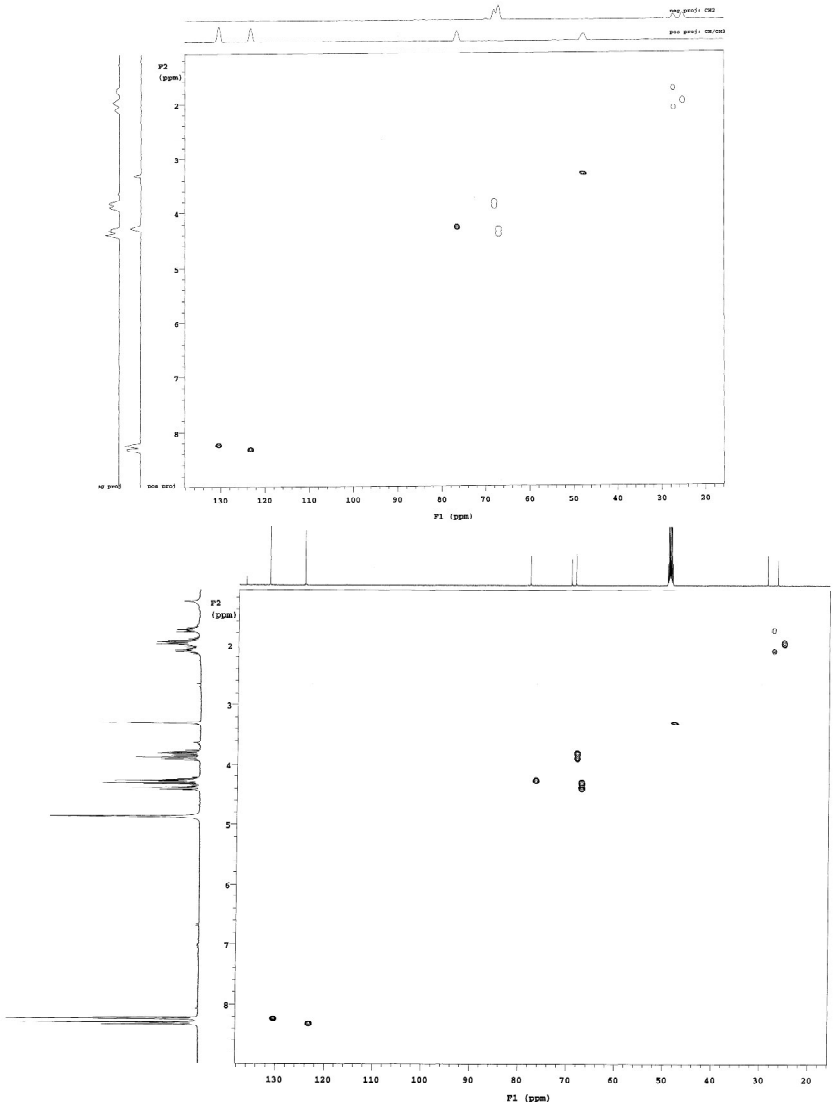
S5
^{1}H-NMR Spectrum of Compound 1



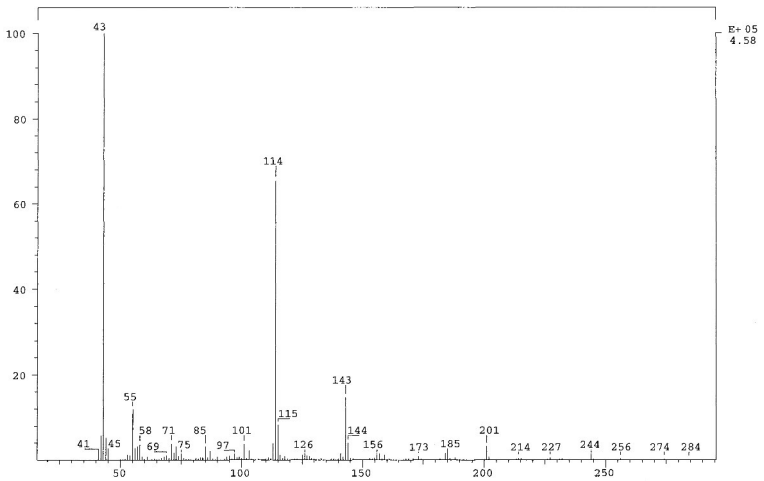
S6
HMBC Spectrum of Compound 1



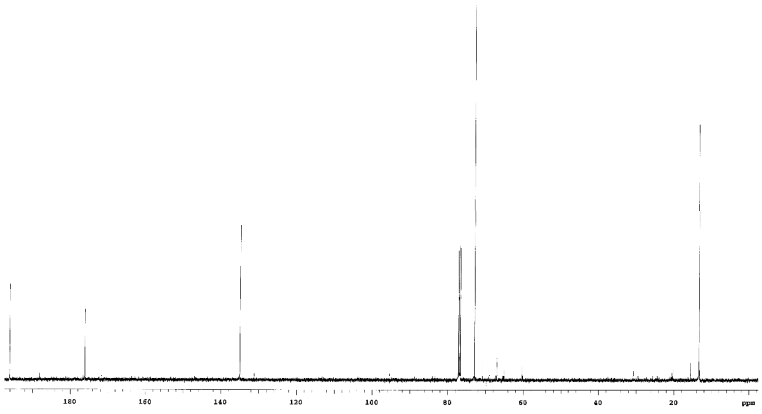
S7
COSY Spectrum of Compound 1



S8
HSQC Spectrum of Compound 1



S9
(-)-ESI MS Spectrum of Compound 5



S10
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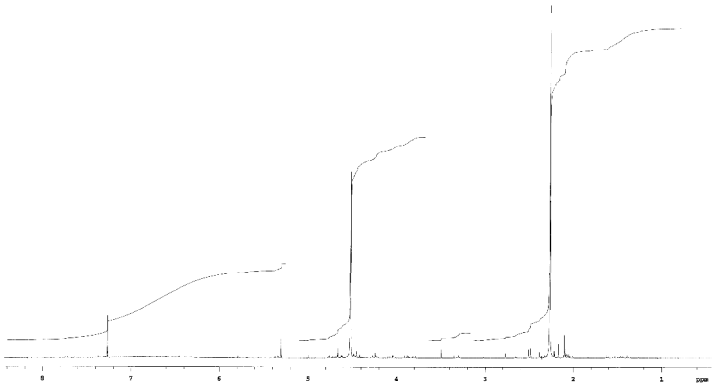

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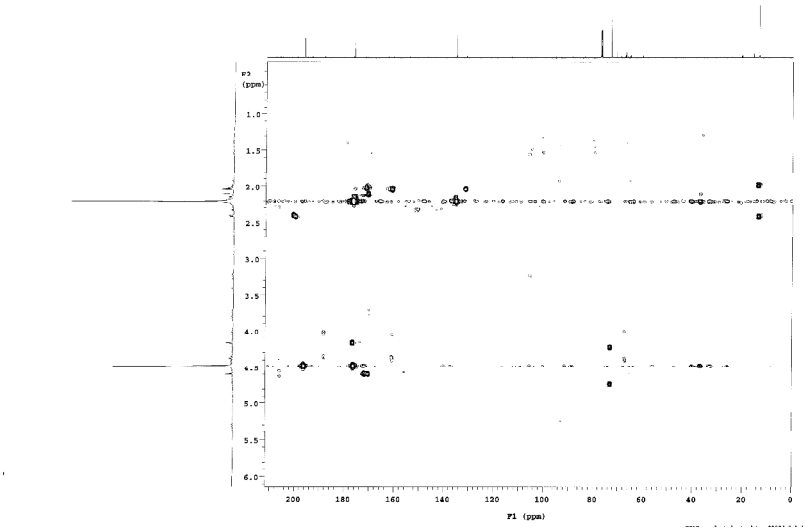

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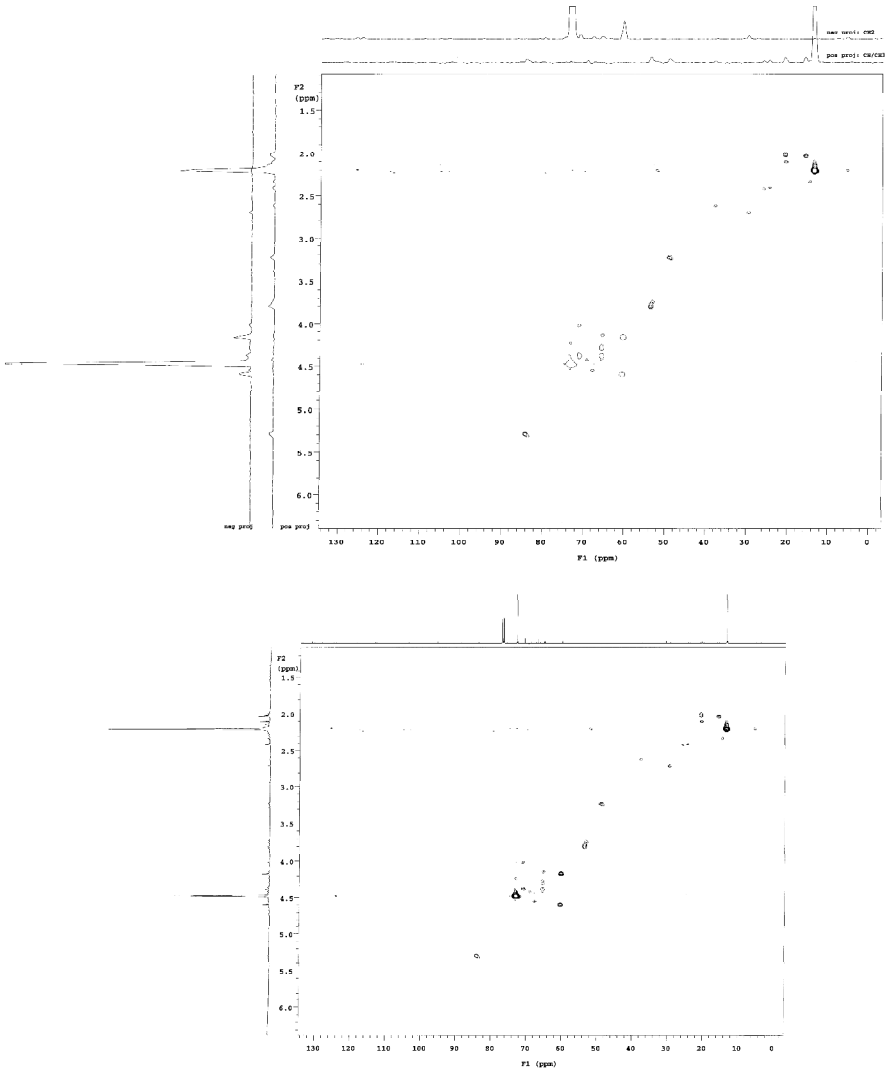

S13
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