FUNGITOXIC ACTIVITY OF TWO SESQUITERPENE COMPOUNDS FROM DRECHSLERA CYNODONTIS AGAINST PLANT PATHOGEN FUNGI.

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ABSTRACT

Two, sesquiterpenes; Dihydrobipolaroxin (1) and Sorokinianin (2). have been isolated from the culture filtrates of the fungus *Drechslera cynodontis*. This paper describes the isolation and structure elucidation of the two chemical compounds by EL-MS, 1H-NMR, 13C-NMR, DEPT, COSY, HMQC and HMBC spectra techniques that led to characterization of the two novel compounds; Dihydrobipolaroxin and Sorokinianin. The results obtained for identification of the compound 1 by EL-MS suggesting the molecular formula $C_{18}H_{28}O_4$. The structure showed consistent with the structure derived by13C-NMR and DEPT spectrum and the structure was confirmed in full agreement with those reported for dihydrobipolaroxin sesquiterpene by Sugawara *et al.* 1985. The antifungal activity of culture filtrate of *Drechslera* isolate on the mycelial growth showed significant reduction in mycelial growth and spore germination of all plant pathogens under study. Data showed highly effective growth inhibition against *A. solani*, *F. oxysporum* and *S. sclerotiorum* up to 66.7%, while decreased the other fungal growth by 44.4–51.1% at concentration of 100 μg/ml. Compound 1 caused higher reduction in spore germination of A. solani (8%) and F. solani (77%) at 100 μg/ml concentration.

For compound (2) The EI-MS showed a molecular ion peak [M+1]+ at m/z 309 suggesting the molecular formula $C_{18}H_{28}O_4$. The detailed 1H-NMR and 13C-NMR data were consistent and compound identified as Sorokinianin and in agreement with results reported by Nakajima *et al.* (1994). Compound 2 (Sorokinianin) decreased the fungal growth at concentration of 100 μg/ml in all tested fungi which ranged from 22.2% growth inhibition in *R. solani* to 61.1% in *F. solani*. Compound 2 was highly effective in suppressing spore germination of F. solani (75%) at 100 μg/ml concentration. Data of experiments done to determine the minimum inhibitory concentrations (MIC) for fungal growth indicated that maximum concentration (100 μg/ml) of both compounds was not sufficient for inhibiting completely the growth of all tested fungi. All tested compounds recorded higher MIC values (>100 μg/ml). The MIC for spore germination Compounds 1 and compounds 2 had less inhibitory effect on spore germination of all tested fungi, they recorded- higher MIC values (>100 μg/ml).
Keywords: *Drechslera cynodontis*, sesquiterpenes, antifungal activity, plant pathogenic fungi

INTRODUCTION

Toxins produced by phytopathogenic fungi have assumed great importance because of their involvement in several plant diseases. Most of the plant pathogenic fungi produce toxins in culture and in their hosts. The

virulence of the plant pathogen may depend on its capability to synthesize one or more toxins. The chemical nature of these toxins ranges from low molecular weight compounds includes all classes of natural products such as terpenes, chromanones, butenolides, pyrones, macrolides, aromatic derivatives, amino acids etc., to high molecular compounds such as proteins, glycoproteins and polysaccharides. In some cases, toxins have been used to obtain products for plant protection or, by genetic selection, plants resistant to specific disease (Heiser *et al.* 1998). Because phytotoxins are biologically active compounds, various applications employing these metabolites have been attempted. Due to their role in disease development, some phytotoxins have been proposed as potential markers for screening disease resistant plants. Phytotoxins could also be used as potential fungicides and herbicides. Since phytotoxins are toxic not only to crop plants, but also to weeds, herbs, etc. Overall, phytotoxins appear to have an important role in the invasion of the plant tissue, how they are produced, how they interact with the plant defense mechanisms, will bring understanding of the plant-pathogen relationship that would ultimately lead to designing better methods to increase plant resistance against fungal pathogens (Buiatti and Ingram, 1991). The search for new products for the pharmaceutical and agrochemical industries is an on-going process that requires continual optimization. Previously, the screening of 10000 natural products resulted in one commercial products. In the advent of combinatorial chemistry, this relationship changed. Presently, the screening of 100000 structures day from combinatorial chemistry together with the natural products screened yields less than one commercial product year. Its development takes approximately 12 yr and costs ~ $ 350 M. Considering that 6 out of 20 of the most commonly prescribed medications are of fungal origin and only ~ 5% of the fungi have been described. Fungi offer an enormous potential for new products (Gloer, 1997). The objectives of this study are: 1) Extraction of fungitoxic metabolites from culture filtrates of Drechslera cynodontis, 2) Effect of isolated compounds on mycelial growth and spore germination of various phytopathogenic fungi, 3) Determination of the minimum inhibitory concentration (MIC) of the active substance's.

MATERIALS AND METHODS

Drechslera isolate

An isolate of *Drechslera cynodontis* (Marignoni) Subram & B.L. Jain. was obtained from Assiut University Mycological Centre (AUMC), Egypt. The fungus *D. cynodontis* is a natural fungal pathogen on Bermuda grass.

Plant pathogens

Botrytis fabae, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotinia sclerotiorum and *Sclerotium cepivorum* were isolated from different crops on potato dextrose agar (PDA) and Czapek agar media. The plates were incubated at 26 ± 2°C or 20 ± 2°C for 7 days. Cultures were purified using single spore or hyphal tip techniques. Pure cultures were identified by mycologists from the Assiut University Mycological Centre

(AUMC), Egypt. The fungal cultures were maintained on half-strength potato dextrose agar slants in small vials under mineral oil at 4°C. Fungal strains of *Alternaria solani* and *Botrytis cinerea* were obtained from the Plant Pathology Institute, Agricultural Research Center, and Giza, Egypt.

Production of culture filtrates

For toxin production, small pieces from mycelial mats of 10-day-old cultures of D. cynodontis, grown on PDA Petri dishes, were transferred to 500 ml flasks containing 200 ml of modified M-1-D medium described by. (Pinkerton and Strobel, 1976) The cultures were incubated under static conditions at 27 ± 2°C in the dark for 4 weeks. The culture fluid was obtained by filtrating through three layers of cheesecloth, and then two layers of the Whatman No.1 filter paper.

Extraction of the metabolites

Culture filtrates of D. cynodontis were concentrated to 10% of their original volume by using freeze drying. The concentrated filtrate was extracted with chloroform followed by ethyl acetate, then n-butanol using separating funnel (3 extractions each using 0.3 volume of organic solvent per volume of filtrate). The chloroform, ethyl acetate and n-butanol extracts were filtered over anhydrous Na_2SO_4. All the organic fractions separated by solvent extraction were subjected to dryness using rotary evaporator to remove any traces of solvents and to obtain the final residues. The residues were weighed, and collected in vials using methanol. Residues were named as obtained, fraction A (chloroform extract), fraction B (ethyl acetate extract) and fraction C (butanol extract).

Isolation and purification of metabolites

Thin layer chromatography (TLC): The chloroform, ethyl acetate and butanol extracts were subjected to thin layer chromatography (TLC) using different solvent systems. The thin layer chromatography sheets used were TLC aluminium sheets, 20 × 20 cm, silica gel 60, ref: 1.05553., Merck, Darmstadt, Germany, and reverse TLC sheets. Bands on TLC sheets were marked under ultra violet (UV) light (254 and 365 nm) or detected by spraying with 5% H_2SO_4 in methanol, followed by heating at 110°C for 10 min till the colours developed.

Preparative TLC: The glass plates of different dimensions were cleaned carefully before use then dried and coated with silica gel GF 254 LR previously slurred with distilled water. The coated plates were left to dry at room temperature then activated in hot-air oven at 100-110°C for one hour and immediately transferred to a dessicator to cool and kept until used. The samples were spotted at 1.5 cm from the lower edge using a capillary tube. The running length for the solvent was from 9.5 to 10 cm. The developed plates were air dried and the metabolite was exposed to either 254 or 365 nm UV light. Each band was carefully cut off, and each band with silica gel was re-extracted in hot methanol. The solution was further filtered through a 0.2 mm filter to eliminate the silica gel, then dried by flash evaporator, and weighed. The purified metabolites were used for chemical analysis.

Column Chromatography (CC): Gravitatory columns were used in the first stage of purification and a smaller column in the final stage. All columns were packed with silica gel (Merck, Kieselgel 60, 0.063-0.200 mm, 70-230 mesh

ASTM, ref: 1.07734.1000, Darmstadt, Germany). The elution of chloroform, ethyl acetate and n-butanol extracts were developed with suitable solvents in the order of increasing polarity.

Identification of bioactive compounds

Mass Spectra (MS): The Electron Impact Mass Spectra (EI-MS) had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

Nuclear Magnetic Resonance (NMR): 1H-NMR and 13C-NMR spectra were recorded at 500 MHz in dimethyl sulfoxide (DMSO). The same solvent was used as internal standard. Carbon multiplicities were determined by Distortionless Enhancement by Polarization Transfer (DEPT). Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Connectivity (HMBC) NMR experiments were performed using Bruker microprograms.

Effects on mycelial growth of different phytopathogenic fungi

The antifungal activity of pure compounds to tested fungi was investigated using the following mycelial radial growth bioassay. Concentrations were prepared in the range 50 - 100 µg/ml from pure compounds. The test was carried out by growing each fungal species in Petri dishes containing 20 ml of potato dextrose agar (PDA) amended with concentrations of 50, 75 and 100 µg/ml of pure compounds (control plates containing only DMSO). The centre of each PDA plate was inoculated with one fungal disc (5 mm diameter) from edges of fungal solid cultures of the respective fungus. The plates were incubated at 24 ± 2ºC for 1-2 weeks, depending on the fungal species. The antifungal activity was evaluated by measuring the diameter of test and control colonies in millimeter. Each assay was repeated at least three times. The percentage of inhibition was calculated on the basis of growth in the control plates using the expression:

$$\text{\% Mycelial inhibition} = (X - Y / X) \times 100$$

Where; X and Y are the average diameters of mycelial colonies in control and treated fungi sets.

Effects on spore germination of different phytopathogenic fungi

The assays were carried out with *A. solani, B. cinerea, B. fabae, F. oxysporum* and *F. solani*, as test fungi. Cultures of the fungi were grown on PDA and/or potato carrot agar (PCA) media for 7 – 10 days at 25 ± 2°C until well sporulated except A. solani. The spores were harvested by adding 10 ml of sterile water and aseptically dislodging the spores with a sterile inoculating loop. Spores suspensions were aseptically filtered through sterile cheesecloth to remove mycelial debris. pure compounds were diluted in sterile distilled water to produce twofold serial concentrations 50, 75 and 100 µg/ml and 1 ml portions of each concentration were add to test tubes. Spores suspended in distilled water were diluted in potato dextrose broth to produce the inoculum of 1.0×10^5 spores/ml by using haemocytometer slide. Portions of 1 ml of a particular fungal spore suspension were added to the tubes that contained pure compounds or distilled water (control). These tubes were then incubated at 24 ± 2ºC for 24 hours (control tubes containing only DMSO). The

percentage of spore germination was calculated in 100 spores from 5 microscopic fields. Three tubes were prepared for each treatment and the data was recorded as the mean of three replicates. Sporulation was induced in A. solani by growing it in culture plates of PMDA medium (15 g instant mashed potato mix, 15 g dextrose, 23 g agar and 1.5 l water) for 10 to 14 days at room temperature under constant or diurnal light, then cutting the culture medium into 4-cm strips and placing them in 250 ml sterile water in flasks. Vigorously shake the flasks for 1 min, let stand for 10 min, then spread 1.5 ml of the fluid on fresh plates of this medium and incubate under constant fluorescent light at 20ºC or in diurnal light if above 23ºC. Transferring small block from a 2-day-old PDA colony of A. solani to corn meal agar medium and incubating under light for 4 hours, then in the dark at 18ºC induces sporulation within 12 hours (Dhingra and Sinclair 1995).

Determination of the Minimum Inhibitory Concentrations (MIC)

The MIC was determined as the lowest concentration of pure compounds that completely inhibited visible fungal growth. Also, the MIC was determined as the lowest concentration of pure compounds that induced no germination of fungal spores or inhibited 90% or more of spore germination.

RESULTS

Extraction procedure:

Culture filtrates of *Drechslera cynodontis* were concentrated in a rotary evaporator at 45°C to a small volume (10% of original volume). Successive extraction was done in a separating funnel with chloroform, ethyl acetate and n-butanol till complete exhaustion. Each fraction was dried over anhydrous sodium sulphate, concentrated in a rotary evaporator, placed in a dessiccator and weighed. Chloroform (9.5 g), ethyl acetate (6.5 g) and n-butanol (15 g), respectively. The residues were kept in a refrigerator for further investigation.

Application of the chloroform extract:

The chloroform extract of *D. cynodontis* culture filtrates (9.5 g) was applied as a band to the top of a glass column packed with slurry of silica gel 60 in chloroform. Elution was performed using chloroform, then mixtures of chloroform-methanol with increasing polarity (99:1, 98:2). Fractions of 100 ml were collected. Each fraction was concentrated to 5 ml volume and monitored by TLC on silica gel 60 plates using chloroform–methanol (9.5:0.5 and 9:1) as solvent systems, and vanillin-H_2SO_4 as spraying reagent. Similar fractions were pooled together, concentrated to dryness, placed in a dessiccator and weighed. The results are recorded in Table (1).

Table 1: Chromatographic fractionation of the chloroform extract of *D. cynodontis* culture filtrates

Fraction No.	Eluent	Constituent
1 – 4	CHCl₃ (100%)	-
5 – 14	CHCl₃ (100%)	one major spot (compound 1)
15 – 20	CHCl₃ – MeOH (99:1)	-
21 – 26	CHCl₃ – MeOH (98:2)	one major spot (compound 2)
27 – 35	CHCl₃ – MeOH (97:3)	-
36 – 40	CHCl₃ – MeOH (96:4)	-

Combined fractions (5-14): Fractions (5-14) were applied as a band to the top of a glass column packed with a slurry of silica gel 60 in CHCl3. Fractions of 20 ml were collected and monitored by TLC on silica gel 60 plates using CHCl3–MeOH (9.5:0.5 and 9:1) as solvent systems and vanillin-H2SO4 as a spraying reagent. Subfractions 6-8 were pooled together to afford pure compound 1 (Rf value = 0.37 in CHCl3–MeOH 9:1), which was concentrated to dryness and weighed (200 mg).

Combined fractions (21-26): Fractions (21-26) from the first column were applied as a band to the top of a glass column packed with a slurry of silica gel 60 in CHCl3. Elution was performed using CHCl3 then mixtures of CHCl3–MeOH with increasing polarity (1%, 2% and 3%). Fractions of 20 ml were collected and monitored by TLC on silica gel 60 plates using the same solvent system and spray reagent previously used. Sub-fractions 3-6 were concentrated and the major component was purified over a preparative TLC using silica gel GF 254 LR, developed in chloroform–methanol (9:1). The band of the major component was located using 254 nm UV light and scraped with a spatula. The component was extracted from powdered silica in methanol and the solvent evaporated to give a pure compound 2 (60 mg).

Identification of the isolated compounds

Compound (1): The EI-MS showed a molecular ion peak [M]+ at m/z 264 and thus suggested the molecular formula C15H20O4. 13C-NMR (Table 2) and DEPT spectrum confirmed the presence of 2 methyl groups at δC 11.2 and 23.1 ppm, 3 methylene at δC 46.5, 61.3 and 109.8 ppm, 5 methine at δC 42.3, 66.5, 123.4, 127.9 and 139.5 ppm and 5 quaternary carbons at δC 36.4, 76.4, 154.2, 163.6 and 197.5 ppm.

The structure was deduced to be bicyclic based on the calculated double bond equivalence (DBE = 6) and the presence of 3 double bonds and one carbonyl carbon. 13C-NMR signal at δ 197.5 confirmed the presence of a keto group. In 13C-NMR spectrum six signals were observed in the olefinic region. Two carbon signals at δC 127.9 and 139.5 were attributed to C-1 and C-2 respectively, signals at δC 123.4 and 163.6 were assigned to C-9 and C-10 respectively and the characteristic signals for exomethylene group at δC 154.2 and 109.8 were assigned to C-11 and C-13 respectively. Besides, DEPT spectrum confirmed the presence of a primary hydroxyl at δC 61.3 (C-12), a secondary hydroxyl at δC 66.5 (C-3) and a tertiary hydroxyl at δC 76.4 (C-7). 1H-NMR and 13C-NMR were in full agreement with those reported for dihydrobipolaroxin sesquiterpene (Sugawara *et al.* 1985). The structure of

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compound 1 (Dihydrobipolaroxin) was further substantiated by complete assignments of HMBC, HMQC and COSY experiments spectra, which have not been previously reported for it.

Table (2): NMR spectral data of compound 1

Carbon number	δ 13C-NMR (J HZ)	δ 1H (multiplicity, J HZ)
1	127.9 CH	6.23 br.s.
2	139.5 CH	6.23 br.s.
3	66.5 CH	(3.95 d, J=14.4)
4	42.3 CH	1.52 m
5	36.4 C	------------
6	46.5 CH_2	1.86 m
7	76.4 C	------------
8	197.5 C	-----------
9	123.4 CH	5.7 s
10	163.6 C	-----------
11	154.2 C	----------
12	61.3 CH2	3.78 s
13	109.8 CH2	5.15 s & 5.18 s
14	23.1 CH3	1.32 s
15	11.2 CH3	0.93 s

In HMBC spectrum the proton at δH 6.23 (1 H, br.s., H-1) showed cross peaks with C-3, C-5, C-9 and C-10, the proton at δH 5.7 (1 H, S, H-9) showed cross peaks with C-5, C-7 and C-1.The presence of the exomethylene group in the side chain was confirmed from COSY and HMBC as its two protons at δH 5.15 and 5.18 ppm showed cross peaks with the proton at δH 3.78 (2 H, S, H-12) (COSY) and with C-7, C-11 and C-12 (HMBC). EI-MS (Fig. 2), 1H-NMR (Fig. 3), 13C-NMR (Fig. 4), DEPT (Fig. 5), COSY (Fig. 6), HMQC and HMBC spectral analyses concluded the following structure:
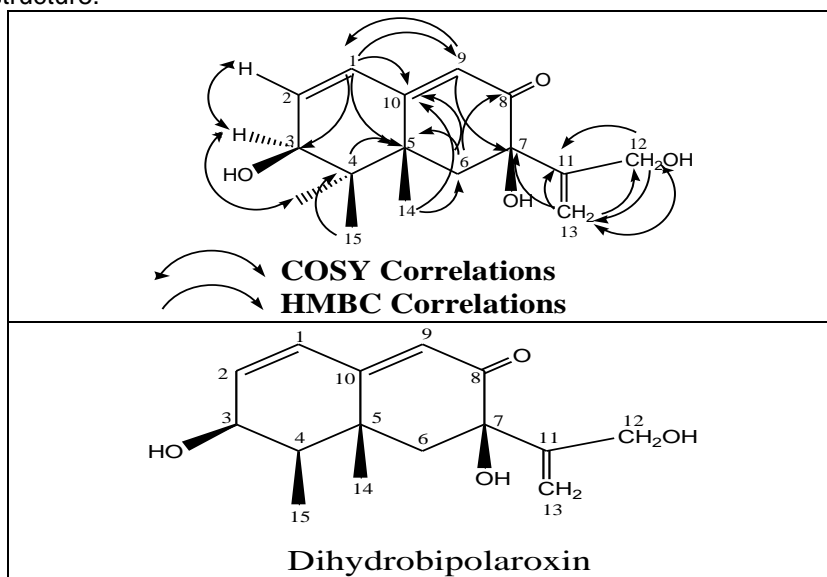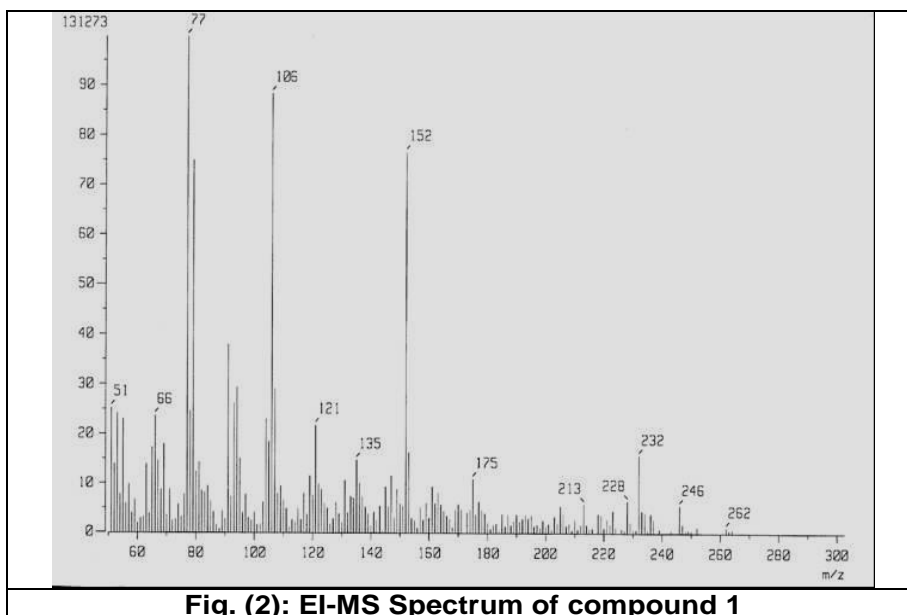

Fig. (1): COSY and HMBC Correlations of compound 1

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Fig. (2): EI-MS Spectrum of compound 1

Compound (2): The EI-MS showed a molecular ion peak [M+1]+ at m/z 309 suggesting the molecular formula C18H28O4. The structure was suggested to be tricyclic based on the calculated double bond equivalence (DBE = 5) and the presence of one double bond and one carbonyl carbon (Table 3).
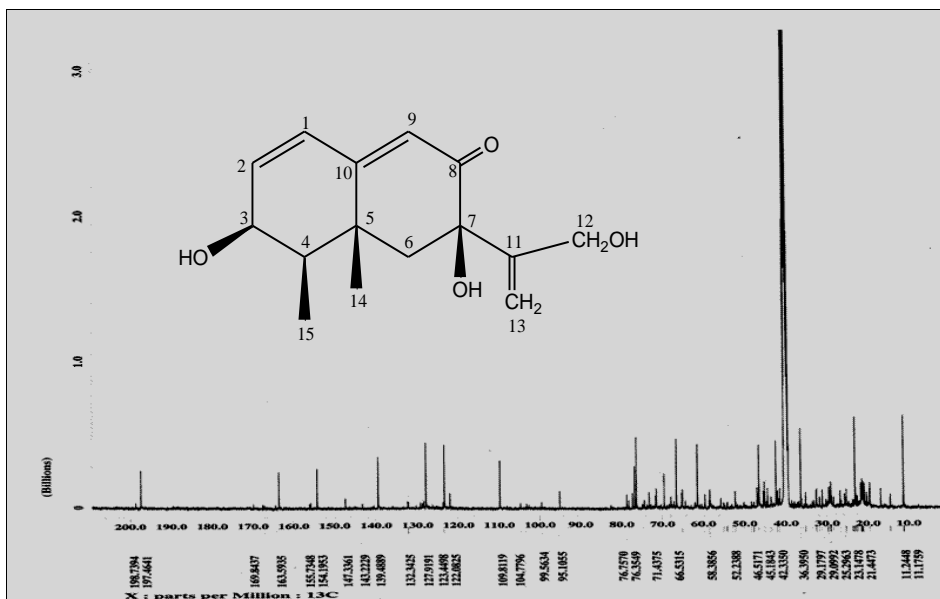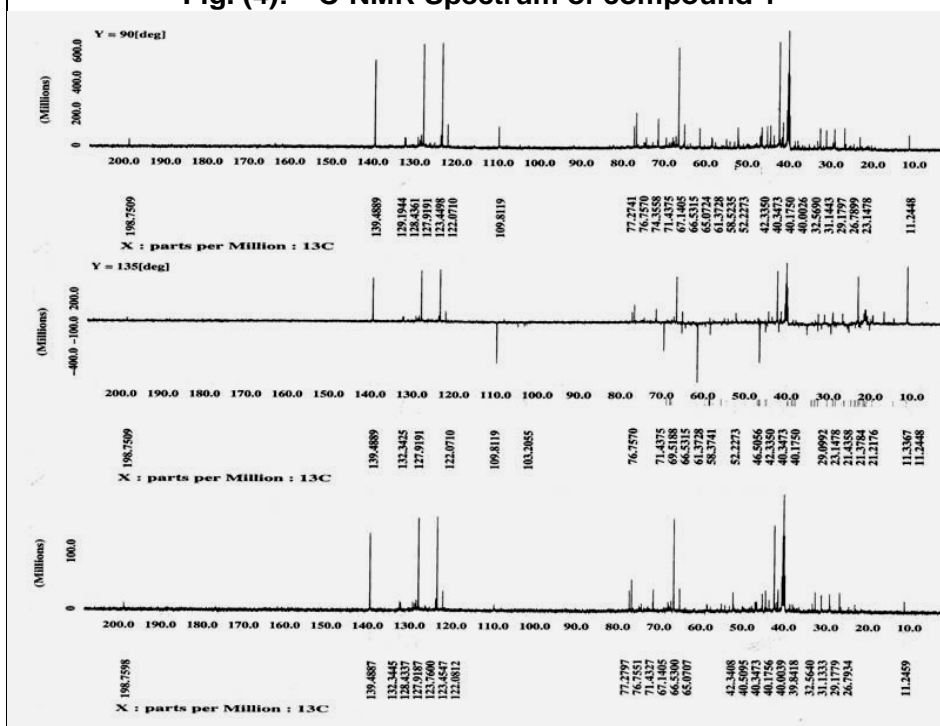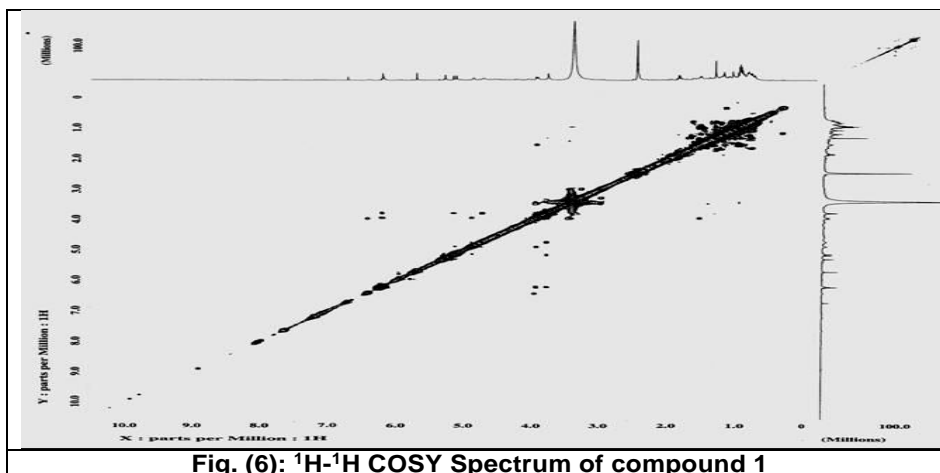

Fig. (3): ¹H-NMR Spectrum of compound 1

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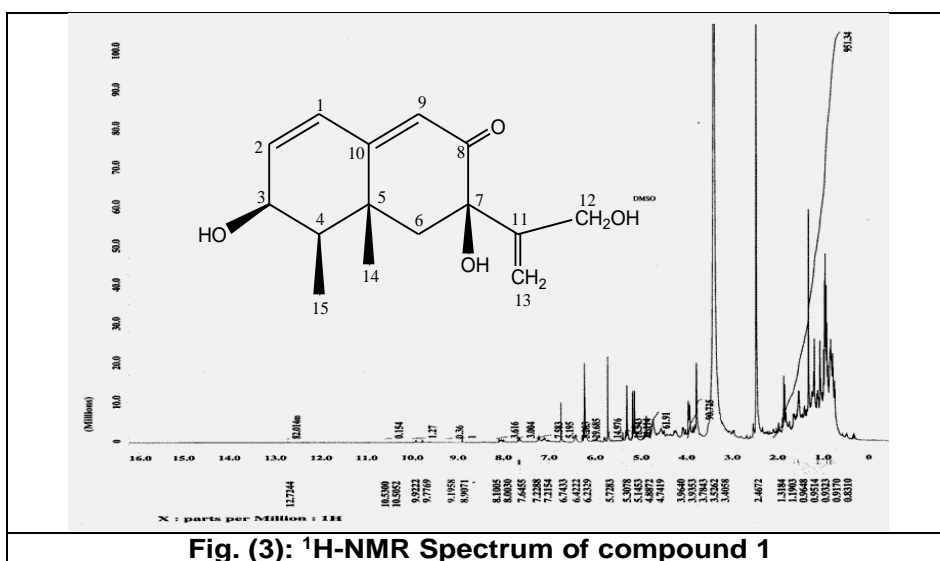
Fig. (4): ^{13}C-NMR Spectrum of compound 1

Fig. (5): DEPT Spectrum of compound 1

Fig. (6): ¹H-¹H COSY Spectrum of compound 1

Table (3): ¹H- and ¹³C-NMR spectral data of compound 2

Carbon number	δ 13C-NMR (J HZ)	δ 1H (multiplicity, J HZ)
1	48.3	----------------
2	44.2	----------------
3	24.7	----------------
4	49.4	----------------
5	39.2a	2.6 br.s.
6	48.3	----------------
7	159.2	----------------
8	59.6	----------------
9	31.2	----------------
10	20.6b	0.77 (d, J=6.9)
11	20.9b	0.88 (d, J=6.9)
12	107.3	4.78 s 5.1 s
13	82.3	4.36 ddd (J=6.0, 6.9, 10)
14	61.5	-----------------
15	22.1	0.91 s
1\	37.8	-----------------
2\	67.0	-----------------
3\	177.1	-----------------

ᵃ - under solvent peak
ᵇ - may be interchanged

An absorption band at 1770 cm-1 (IR spectrum) and a C=O signal at δC 177.1 ppm indicated a γ–lactone ring. Besides the characteristic signals for the exomethylene group at δC 159.2 and 107.3 were assigned to C-7 and C-12 respectively. The presence of isopropyl group was confirmed from the two methyl signals at δH 0.77 (3 H, d, J=6.9, H-10) and δH 0.88 (3 H, d, J=6.9, H-11), both have the same J value i. e. most possibly splitted by the same proton. The detailed 1H-NMR and 13C-NMR data were consistent with those of Sorokinianin (Nakajima *et al.* 1994). EI-MS (Fig. 7), 1H-NMR (Fig. 8) and 13C-NMR (Fig. 9) spectral analyses concluded the following structure:
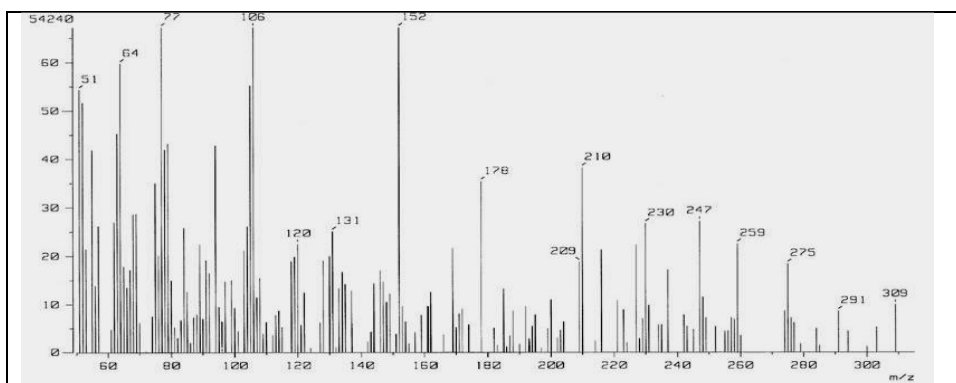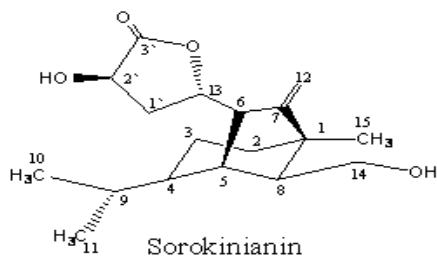
Sorokinianin

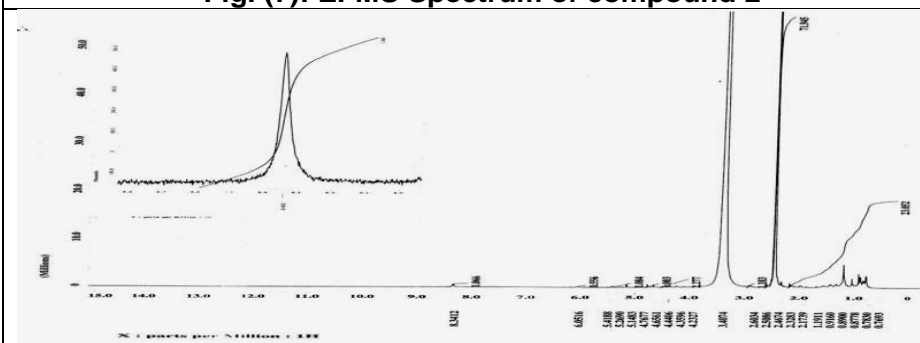**Fig. (7): EI-MS Spectrum of compound 2**
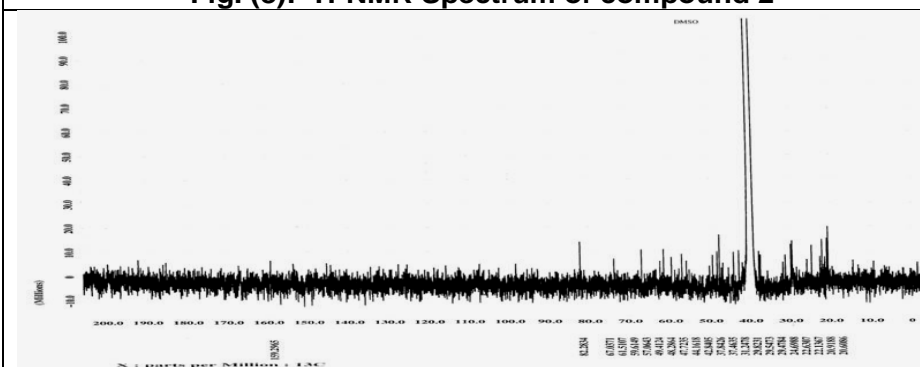

Fig. (8): ¹H-NMR Spectrum of compound 2

Fig. (9): 13C-NMR Spectrum of compound 2

Antifungal activity of culture filtrates on the mycelial growth: Data in Tables (4) show that compound 1 (Dihydrobipolaroxin) was highly effective growth inhibitor against *A. solani, F. oxysporum* and *S. sclerotiorum* (66.7%), while decreased the other fungal growth by 44.4 – 51.1% at concentration of 100 µg/ml. Compound 2 (Sorokinianin) decreased the fungal growth of all tested fungi which ranged from 22.2% (on *R. solani*) to 61.1% (on *F. solani*) at concentration of 100 µg/ml.

Table (4): Antifungal activity of pure compounds isolated from Drechslera cynodontis on the mycelial growth of tested fungi

Fungi	Conc. µg/ml	Inhibition of mycelial growth (%)*			
		Compound 1	Compound 2	Control	DMSO
Alternaria solani	50	36.7	22.2	0	0
	75	55.6	33.3	0	0
	100	66.7	55.6	0	0
Botrytis cinerea	50	16.7	11.1	0	0
	75	38.9	20	0	0
	100	50	44.4	0	0
Botrytis fabae	50	5.6	5.6	0	0
	75	33.3	22.2	0	0
	100	51.1	44.4	0	0
Fusarium oxysporum	50	11.1	11.1	0	0
	75	33.3	20	0	0
	100	66.7	55.6	0	0
Fusarium solani	50	16.7	20	0	0
	75	38.9	50	0	0
	100	50	61.1	0	0
Rhizoctonia solani	50	0	0	0	0
	75	44.4	11.1	0	0
	100	44.4	22.2	0	0
Sclerotinia sclerotiorum	50	41.1	11.1	0	0
	75	51.1	33.3	0	0
	100	66.7	57.8	0	0
Sclerotium cepivorum	50	33.3	33.3	0	0
	75	36.7	55.6	0	0
	100	48.9	58.9	0	0

* % Mycelial inhibition = (X - Y / X) ×100 Where; X and Y are the average diameters of mycelial colonies in control and treated fungi sets. The method of (Dhingra and Sinclair 1995) was used to grow and sporulated fungi

Antifungal activity of culture filtrates of *Drechslera* isolates on spore germination

Data in Table (6) show that compound 1 (Dihydrobipolaroxin) highly decreased spore germination of *A. solani* and *F. solani* by 80 and 77%, while it inhibited spore germination of the other fungi by 34% to 61% at concentration of 100 µg/ml. Compound 2 (Sorokinianin) was highly effective in suppressing spore germination of *F. solani* (75%) at conc.100 µg/ml, while it inhibited the spore germination of the other four fungi by 43% to 60%.

Table (5): Antifungal activity of pure compounds isolated from Drechslera cynodontis on spore germination of tested fungi

Fungi	Conc. µg/ml	Inhibition of spore germination (%)*			
		Compound 1	Compound 2	Control	DMSO
Alternaria solani	50	60	20	0	0
	75	75	30	0	0
	100	80	60	0	0
Botrytis cinerea	50	19	26	0	0
	75	21	45	0	0
	100	34	59	0	0
Botrytis fabae	50	28	40	0	0
	75	49	40	0	0
	100	61	50	0	0
Fusarium oxysporum	50	10	11	0	0
	75	23	27	0	0
	100	56	43	0	0
Fusarium solani	50	50	50	0	0
	75	61	60	0	0
	100	77	75	0	0
	50	60	20	0	0

* **The percentage of spore germination was calculated in 100 spores from 5 microscopic fields using haemocytometer slide**

Determination of the minimum inhibitory concentrations (MIC)

The MIC for fungal growth: Data in Table (5) show that the maximum concentration (100 µg/ml) of any compounds was not sufficient for inhibiting completely the growth of tested fungi. All tested compounds recorded higher MIC values (>100 µg/ml).

The MIC for spore germination: Compounds 1 and compounds 2 had less inhibitory effect on spore germination of all tested fungi, they recorded higher MIC values (>100 µg/ml).

Table (6): Effect of MIC* of pure compounds from D.cynodontis on the mycelia growth and spore germination of the tested fungi

Compound	Compound 1	Compound 2
Alternaria solani	>100**	>100
Botrytis cinerea	>100	>100
Botrytis fabae	>100	>100
Fusarium oxysporum	>100	>100
Fusarium solani	>100	>100
Rhizoctonia solani	>100	>100
Rhizoctonia solani	>100	>100
Sclerotium cepivorum	>100	>100

* **MIC =minimum inhibitory concentrations ** Concentration 100 µg/ml.**

DISCUSSION

Two compounds (dihydrobipolaroxin and sorokinianin) isolated from culture filtrates of the fungus *D. cynodontis*. This is the first report of separation of dihydrobipolaroxin from this fungus. It was separated before from *Bipolaris cynodontis* (Sugawara *et al.* 1985). This is the first time to carryout the 2D-NMR experiments (HMBC, HMQC and COSY) for this compound. Also, this is the first report of isolation of sorokinianin from *D. cynodontis*. It was isolated before from *Bipolaris sorokiniana* (Nakajima *et al.*

1994). Dihydrobipolaroxin and sorokinianin belong to sesquiterpenes. The sesquiterpenes are a large family of C15-isoprene natural products found in higher and lower plants, microbes and some marine organisms. Approximately 5000 sesquiterpenes have been reported. Mono-, bi-, tricyclic sesquiterpenes are all common in plants and fungi. Many have biological activity, including antimicrobial, antitumor, and cytotoxic properties. In plants, they play important ecological roles in interactions with insects and microbes and act as attractants, deterrents, antifeedants and phytoalexins. Sesquiterpenes are key components of many essential oils, which are important commercially for the flavour and fragrance industries. Sesquiterpenes from fungal pathogens are sometimes active as phytotoxins, i. e., compounds that break down host tissues and release nutrients for the pathogens (Fraga 1999).

Sesquiterpenes belong to the largest class of natural products, terpenes (also known as terpenoids or isoprenoids). Of the approximately 25,000 terpene structures reported, very few have been investigated from a functional perspective. All the two compounds were tested for inhibitory activity against some phytopathogenic fungi. Compound 1 (Dihydrobipolaroxin) was highly effective growth inhibitor against *A. solani, F. oxysporum* and *S. sclerotiorum* (66.7%), while compound 2 (Sorokinianin) decreased the fungal growth of all tested fungi which ranged from 22.2% (on R. *solani*) to 61.1% (on *F. solani*) at concentration of 100 µg/ml. Compound 1 highly decreased spore germination of *A. solani* and *F. solani* by 80 and 77%, while compound 2 was highly effective in suppressing spore germination of *F. solani* (75%) at concentration of 100 µg/ml,(inhibiting the spore germination of other fungi by 43% to 60%).

Species of *Bipolaris, Drechslera, Exserohilum*, and *Curvularia* constitute a group of taxonomically related and ecologically similar deuteromycetes (mitosporic fungi) that are important plant pathogens or common saprophytes throughout the world. The first three of these genera were segregated from *Helminthosporium* in several revisions from 1930 to 1974, and some species of *Bipolaris* and *Curvularia* share the same teleomorph (Pratt 2006). The generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia germinating by one germ tube from each end (bipolar germination). (The former genus *Helminthosporium* was divided into three anamorphic genera: *Bipolaris, Drechslera,* and *Exserohilum* with the teleomorphic stages *Cochliobolus, Pyrenophora*, and *Setosphaeria*, respectively.) *B. sorokiniana* is characterized by thick-walled, elliptical conidia (60–120 µm × 12–20 µm) with five to nine cells. In axenic culture, the mycelium is composed of hyphae interwoven as a loose cottony mass and appears white or light to dark grey depending on the isolates. The fungus is differentiated from other members of the *Bipolaris* genus on the basis of morphological features of conidiophores and conidiospores (Kumar *et al.,* 2002).

The inability of microorganisms to produce large amounts of a toxin or the high costs of purification, represent potential constraints to their practical use as natural pesticides. Their chemical syntheses or the chemical

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synthesis of the active moiety could overcome those limitations. Unfortunately, the natural compounds often have very unusual and complex structures, and by synthesis only very partial structures can be achieved. Several fungal pathogens, especially those belonging to the genera *Drechslera, Alternaria* and *Cochliobolus* produce phytotoxins that are virulence and/or pathogenicity factors. These compounds are active against the same plant species as the fungal pathogens and low (physiological) concentrations of the toxin are able to reproduce symptoms of the natural infections. These plant-specific metabolites have received attention as models for new herbicides. There are many reasons why natural products might be good sources of molecules or molecular templates for pesticides or at least lead to new targets of action (Rimando and Duke 2006). New mechanisms of action for pesticides are highly desirable to fight the evolution of resistance in the target pests, to create or exploit unique market niches, and to cope with new regulatory legislation. Comparatively little effort has been expended on determination of the sites of action of phytotoxins from natural sources, suggesting that intensive study of these molecules will reveal many more novel mechanisms of action (Duke *et al.,* 2000). Correlations of structure-activity are of utmost importance to the knowledge of the structural characteristics of the fungal metabolites and the determination of their active sites, or to hypothesize their chemical transformation to obtain more active, stable or selective compounds.

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تأثير النشاط المضاد للفطريات لمركبين من مركبات السيسكوتربينات معزولين من الفطر دريشسليرا سينودونتس ضد الفطريات الممرضة للنباتات

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تــم فصــل عــدد ٢ مركـب تتبــع مركبــات التربينــات مــن المجموعــة sesquiterpenes وهمــا Dihydrobipolaroxin (١) و Sorokinianin (٢) وذلـك مـن مـن راشـح مـزارع فطـر دريشسـليرا سينودونتس Drechslera cynodontis باستخدام طرق تعريف متقدمة وهى -MS, 1H-NMR, 13C NMR, DEPT, COSY, HMQC, HMBC. والتى أدت إلى تحديد خصائص وتركيب هذين المركبين بدقة. وأظهرت نتـائج تعريف المركبين التفصيلية أن تعريف المركب ١ بواسطة تكنيك EL-MS ان الوزن الجزيئى للمركب هو C18H28O4 وظهرأيضا أن تركيب المركب ١ الذى تم التعرف عليه بواسطة أجهزة NMR-13C and DEPT متفق مع تم الحصول عليه من نتـائج للتعريف على اساس أنه مركب dihydrobipolaroxin sesquiterpene بواسطة الباحث Sugawara ومساعدوه ١٩٨٥. واظهرت التجـارب التى أجريت لتقدير سمية المركب ١ ضد النمو الميسليومى للفطريات الأخرى من خلال أستخدام راشح الفطر D. cynodontis وجود تأثير مثبط بدرجة عالية لنمو الفطريات الممرضة للنباتات تحت الأختبار. وكانت أفضل النتائج المتحصـل عليها هى حدوث تثبيط للفطر التزناريا سولانى والفطر فيوزاريوم اكسيسبورم والفطر أسكليروتينيا اسكليروشيورم بنسبة تثبيط تصل إلى ٦٧% بيما حدث تثبيط لباقى الفطريات بنسب تتراوح من ٤٤- ٥١ % وذلك عند أستخدام التركيز ١٠٠ ميكروجرام/ ١ ملل . كمـا أدى أستخدام المركب ١ إلى حدوث تثبيط لأنبات الجـراثيم فى الأنواع المتجرثمـة من الفطريات تحت الأختبار بنسب تتراوح من ٨% للفطر ألترناريا سولانى إلى ٧٧% فى الفطر فيوزاريوم سولانى.

وبالنسبة للمركب (٢) أظهرت نتائج أختبارات تعريف المركب بتكنيك EI-MS أن الوزن الجزيئى للمركب هو C18H28O4 , كما أظهرت نتائج التحليل المتقدم بأستخدام طرق 1H-NMR- 13C-NMR ثبات نتـائج التعريف الأولية وتطابقها وأن المركب عبـارة عن Sorokinianin وذلك فى تطابق مع مـا تحصل عليه باحثين أخرون مثل الباحث Nakajima ومساعدوه ١٩٩٤.

وفى التجارب التى أجريت لدراسة تأثير المركب ٢ (سوروكينيانين) على نمو الفطريات الممرضة للنباتات أظهرت النتـائج حدوث تثبيط بدرجـه عاليـة لكـل الفطريـات المستخدمة فى الدراسـة عند استخدامه بتركيـز ١٠٠ ميكروجرام / ١ملل وذلك بنسب تثبيط تتراوح من ٢٢% فى نمو فطر رايزوكتونيا سولانى إلى ٦١% فى نمو فطر فيوزاريوم سولانى. كما أدى المركب ٢ إلى حدوث تثبيط عالى لأنبات جراثيم فطر فيوزاريوم سولانى بنسبة ٧٥% عند تركيز ١٠٠ ميكروجرام / ١ملل. كما أظهرت نتائج الأختبارات التى أجريت لتقدير الحد الأدنى المثبط (MIC) لنمو الفطريات المستخدمة فى هذه الدراسة أن التركيز ١٠٠ ميكروجرام / ١ملل فى كلا المركبين ١ ، ٢ هو الحد الأدنى للتركيز الذى يمكن ان يؤثر على نمو وتجرثم هذه الفطريات الممرضة للنبات.