Asian Journal of Chemistry Vol. 19, No. 6 (2007), 4553-4562

Antibacterial Activity of New Polyester Diterpenes from *Euphorbia guyoniana*

ASHRAF A. EL-BASSUONY
Department of Mathematical and Life Sciences, Graduate School of Science Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan
E-mail: ahnnbass@yahoo.com

The dichloromethane extract of the aerial parts of *Euphorbia guyoniana* afforded two new polyesters diterpene, named guyonianin C and D, a rare classes of bicyclic diterpenes. Their structure were established by high-field NMR spectroscopic methods, including DEPT, COSY, NOESY, HMQC and HMBC.

Key Words: *Euphorbia guyoniana*, Euphorbiaceae, Diterpenes, Guyonianin C and D.

INTRODUCTION

Euphorbia is the largest genus of the family Euphorbiaceae with over 1000 species and subdivided into many subgenera and sections, a number of which have been treated as distinct genera[1,2]. Members of this genus are characterized by the presence of a milky irritant latex. The biological activities of the genus including antitumor, antiviral, cytotoxic properties and different vascular effects, which generally attributed to the presence of specific types of diterpenes, both macrocyclic and polycyclic derivatives[3]. Jatrophane and modified jatrophane diterpenoids, which are rare in the genus *Euphorbia*, are potent inhibitors of a membrane protein (so-called P-glycoprotein) pumping cytotoxic drugs out of cells and conferring upon the cells the ability to resist high doses of these drugs[4]. Therefore, the genus has been subjected to numerous chemical studies and led to the isolation of diterpenes[5,6], dimeric diterpenoid[7], diterpene polyesters[8], triterpenes[9], pentacyclic triterpenes[10]. Few sesquiterpenoids and flavonoids were reported from the genus[11,12]. Herein from the methylene extract of *E. guyoniana* Boiss et Reut two new jatrophane diterpenoids, **1** and **2**, named guyonianin C and D have been reported.

EXPERIMENTAL

Optical rotation were measured in $CHCl_3$ with a Perkin-Elemer 2435 polarimeter. IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. NMR spectra were recorded with a Jeol ECA500 spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C). NMR chemical shifts were referenced to

solvent peaks: Δ_H 7.26 (residual $CHCl_3$) and Δ_C 77.0 for $CDCl_3$. FABMS were recorded on a Jeol SX102A mass spectrometer. HPLC was performed in the reverse phase an Knauer instrument, pump type 64, detector: different refractometer (Knauer system), column: RP-8, 250 × 25 mm (Knauer system), flow rate = 17 mL/min, elution with MeOH-H_2O, mixtures.

The aerial parts of *Euphorbia guyoniana* Boiss et Reut. were collected from Ourgula-Algeria in March 2003 and were identified by Dr. A. Chahma, Department of Agriculture, Faculty of Science, University of Ourgula, Algeria. A voucher specimen EG-10019 were deposited at Chemistry Department, University of Mentouri-Constantine, Algeria.

Extraction and isolation: The air-dried and powdered plant (850 g) were extracted exhaustively with CH_2Cl_2-MeOH (1:1) at room temperature. The solvent was distilled under reduced pressure, furnishing a gummy residue (15 g). The residue was submitted to flash column chromatography, being eluted with *n*-hexane, CH_2Cl_2 and MeOH, increasing the degree of polarity. The *n*-hexane-CH_2Cl_2 (25:75) was pre-fractionated by CC a Sephadex LH-20 (6 × 120 cm) eluted with *n*-hexane-CH_2Cl_2-MeOH (6:4:1) gave a complex mixture. The mixture was purified by HPLC (MeOH-H_2O, 70:30, R_2 = 5.6 and 6.0 min) to yield compounds **1** (18 mg) and **2** (16 mg).

Bioassay: The antibacterial activity of guyonianin C and D was determined against Gram-negative strains (*Serratia* sp., *Pseudomonas* sp.) and Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), obtained from culture collection of Bacteriological Laboratory, Department of Botany, Faculty of Science, El-Minia University, Egypt, using Whatman filter paper No. 1, 1 cm. Diameter, disc diffusion assay methods. Five replicates were performed for the two compounds with two concentrations (200 and 400 µg/mL) of each compound were done . Discs were soaked in the test compound for 30 s, evaporated, then overload on the surface of the nutrient agar media cultured with the tested bacterium. All plates were incubated at 30°C for 48 h. Ampicillin (purchased from ADWIC Comp., Egypt) and amoxillin (purchased from ADCO Comp., Egypt) were used as reference compounds

Guyonianin C (**1**): Yellowish brown powder. $[\alpha]^{25}$D-170º (c 1.0, CH_3OH). IR (oil film): 3450, 1738 and 1724 cm^{-1}. ^1H NMR and ^{13}C NMR data (Table-1). MS (positive FABMS): m/z (%) = 820 $[M + H]^+$ (45), 778 $[M + H - AcO]^+$ (10), 736 $[M + H - 2AcO]^+$ (5), positive HRFAB-MS: m/z $[M + H]^+$ calcd. for $C_{43}H_{49}NO_{15}$: 820.344; Found: 820.343.

Guyonianin D (**2**): Greenish brown oil. $[\alpha]^{25}$D -91º (c 0.1, $CHCl_3$). IR (oil film): 1738 and 1727 cm^{-1}. ^1H NMR and ^{13}C NMR data (Table-2). MS (positive FABMS): m/z (%) = 582 $[M + H]^+$ (12), 522 $[M + H - AcOH]^+$ (55), 477 $[M + H - benzoate]^+$ (15), positive HRFAB-MS: m/z $[M + H]^+$ calcd for $C_{33}H_{49}O_9$: 582.309; Found 582.308.

RESULTS AND DISCUSSION

The CH_2Cl_2 extract of the dried aerial parts of *E. guyoniana* was fractionated by column chromatography on silica gel and purified by HPLC to afford two new jatrophane diterpenoids, **1** and **2**. The molecular formula of **1** was established as $C_{43}H_{49}NO_{15}$ on the basis of positive HRFABMS, confirmed by ^{13}C and DEPT NMR (benzene-d_6) analysis, which exhibited an ion peak $[M+H]^+$ at m/z 820.3439. The ^1H and ^{13}C NMR spectra (benzene-d_6, Table-1) of compound **1** revealed the presence of one nicotinate group [δ_H 9.95 br d (1H), 8.53d (1H), 6.77 dd (1H), 8.41 dt (1H); δ_C 164.6 (CO), 127.2 (=C=), 152.0 (CH), 153.8 (CH), 123.1 (CH), 137.1 (CH)], one benzoate group [δ_H 8.21 dd (2H), 7.08 td (2H), 7.14 tt (1H); δ_C 165.5 (CO), 131.0, 130.4 (2 × CH), 128.4 (2 × CH), 132.9 (CH)] and five acetate groups [δ_H 1.67, 1.97, 1.85, 1.69, 2.16; δ_C 169.2, 169.4, 169.6, 169.9, 170.1 (CO) and 20.2, 20.3, 20.7, 21.1, 21.3 (CH_3)]. Additionally, its ^1H NMR exhibited three tertiary methyl groups [δ_H 1.85, 1.21, 0.78], one secondary methyl groups [δ_H 1.06, d, 3H].

The parent diterpene skeleton and its structural fragments were established from series of NMR experiments. ^{13}C NMR and DEPT spectral data of the parent diterpene skeleton revealed the presence of twenty carbon signals: five quaternary carbons, nine methines, two methylenes and four methyl groups. In a ^1H-^1H COSY experiment, the signal at δ_H 5.57 (dd, J = 15.6, 9.6 Hz, H-12) was correlated with two signals at δ_H 5.72 (d, J = 15.6 Hz, H-11) and 3.69 (dq, J = 9.6, 7.1 Hz H-13), suggesting the *trans*-CH_{11}=CH_{12}-$CH_{13}(CH_3)$-moiety, the signal at δ_H 5.47 (br s, H-8) showed a weak correlation with two signals at δ_H 6.13 (br s, H-7) and 5.22 (br s, H-9), indicating the presence of -CH_7(O)-$CH(O)_8$-CH_9(O)- moiety, the signals at δ_H 4.05 (dd, J = 5.5, 5.0 Hz, H-4) was correlated with two signals at δ_H 6.25 (d, J = 5.5 Hz, H-5) and 6.45 (d, J = 5.0 Hz, H-3), which suggested the presence of -CH_3(O)-CH_4-CH_5(O)- moiety, AB system at δ_H 3.35 (d, J = 16.1, H-1a) and 2.34 (d, J = 16.1, H-1b), two olefinic protons at δ_H 5.40 and 5.52. The connectivity of these partial moieties and the positions of the acetate groups were established by HMBC (Fig. 1). In this experiment, H-1β (δ_H 2.34) correlated with C-15 and C-14 as well as C-3, C-4 and C-16, H-1α (δ_H 3.35) with C-2, C-3, C-4 and C-14, H-5 (δ_H 6.25) with C-3 and C-4, H-$17_{a,b}$ with C-5, C-6 and C-7, H-9 (δ_H 5.22) with C-7, C-8, C-10, C-11, C-18 and C-19. The placement of the benzoate group at C-5 was determined from the correlation between H-5 (δ_H 6.25) and the carbonyl carbon of the benzoate (δ_C 165.5) as well as the NOE effects between the two exomethylene protons (δ_H 5.52, 5.40, H-$17_{a,b}$) and H-3',7'. While, the placement of the nicotinate at C-2 was determined from the NOE effects between H-3 (δ_H 6.45) and H-6" (δ_H 6.77). Additionally, the five acetyl carbonyl carbons showed correlation with their acetoxymethine

protons in the HMBC experiment (Table-1 and Fig. 1). The relative stereochemistry of compound **1** was studied through the analysis of the coupling constants and NOESY spectrum. H-4 was used as a convenient reference point, NOE cross-peaks of H-4 with H-1 (δ_H 3.35), H-3, H-7, H-13 and H-3" suggested the α-orientation of those protons and the nicotinate group. The presence of NOEs between H-5/H-8, H-5/15-OAc, H-8/H-19, H-8/H-9, H-12/H-19 and H-12/H-20 suggested the β-orientation of H-5, H-8, H-9, H-12, 15-OAc, H-19 and H-20. These NOE interactions and the relatively small $^3J_{4\text{-}5}$ value (5.5 Hz) are diagnostic for an *exo*-type conformation of the jatropane core[13-15].

TABLE-1
NMR SPECTRAL DATA OF COMPOUND **1** (500 MHz, BENZENE-d_6, 50ºC, δ-VALUES)

Protons	δ_H (J in Hz)**	Carbons*	HMBC	NOESY
1_β	2.34 (d, 16.1)	49.9 (t)	C-3, C-4, C-15, C-16	H-16
1_α	3.35 (d, 16.1)		C-2, C-3, C-4, C-14	H-4 , H-13, H-16, H-20
2		91.0 (s)		
3_α	6.45 (d, 5.0)	79.6 (d)	C-1, C-2, C-15	H-3´, H-3´´, H-4, H-6´, H-16
4_α	4.05 (dd, 5.5, 5.0)	47.9 (d)		H-3, H-1_α, H-7, H-11, H-13
5_β	6.25 (d, 5.5)	71.2 (d)	C-3, C-4, C-1´´	H-8, H-17_b, H-19
6		142.8 (s)		
7_α	6.13 (br s)	68.2 (d)	C-6, C-17, C=O (7-OAc)	H-4, H-11
8_β	5.47 (br s)	70.8 (d)	C-6, C-10, C=O (8-OAc)	H-5, H-9, H-19
9_β	5.22 (br s)	81.3 (d)	C-7, C-8, C-10, C-11, C-18, C-19 C=O (9-OAc)	H-8, H-18, H-19
10		40.8 (s)		
11	5.72 (d, 15.6)	137.4 (d)	C-12, C-13, C-10, C-18, C-19	H-4, H-7, H-13, H-18
12	5.57 (dd, 15.6, 9.6)	129.8 (d)	C-10, C-11, C-13, C-14	H-19, H-20
13_α	3.69 (dq, 9.6, 7.1)	44.3 (d)	C-11, C-12, C-14, C-20	H-1_α, H-4, H-11
14		211.7 (s)		
15		76.8 (s)		
16	1.85 (s)	20.1 (q)	C-1, C-2, C-3	H-1_β, H-1_α
17_a	5.40 (br s)	116.5 (t)	C-5, C-7	H-3´´, 7´´
17_b	5.52 (br s)		C-5, C-6, C-7	H-5, H-3´´, 7´´
18	0.78 (s)	25.7 (q)	C-11, C-10, C-9, C-19	9-OAc, H-9, H-11, H-19

Protons	δ_H (*J* in Hz)**	Carbons*	HMBC	NOESY
19	1.21 (s)	23.3 (q)	C-11, C-10, C-9, C-18	H-5, H-8, H-9, H-12, H-18
20	1.06 (d, 7.1)	20.1 (q)	C-12, C-13, C-14	H-1_{α}, H-12
15-OH	4.54 (br s)			
1´		165.5 (s)		
2´		131.0 (s)		
3´, 7´	8.21 (dd, 7.6, 1.5)	130.4 (d)	C-1´´, C-5´´, C-7´´	H-3, H-17_a, 17_b
4´, 6´	7.08 (td, 7.6, 1.5)	128.4 (d)	C-2´´, C-6´´	
5´	7.14 (tt, 7.6, 1.5)	132.9 (d)	C-3´´, C-7´´	
1´´		164.6 (s)		
2´´		127.2 (s)		
3´´	9.95 (b d, 2.0)	152.0 (d)	C-2´, C-7´, C-5´	H-3, H-4, H-11, H-16
5´´	8.53 (br d, 4.5)	153.8 (d)	C-6´, C-7´, C-3´	
6´´	6.77 (dd, 8.1, 4.5)	123.1 (d)		H-3
7´´	8.41 (dt, 8.1, 2.0)	137.1 (d)	C-1´, C-3´, C-5´	
3-OAc	1.69 (s)	169.9 (s) 21.1 (q)		
7-OAc	1.67 (s)	169.6 (s) 20.2 (q)		
8-OAc	1.85 (s)	169.4 (s) 20.7 (q)		
9-OAc	1.97 (s)	169.2 (s) 20.3 (q)		H-18
15-OAc	2.16 (s)	170.1 (s) 21.3 (q)		H-16

*Multiplicity was determined by DEPT experiments
**Assignments by 1D and 2D spectral data.
***s = quaternary, d = methine, t = methylene, q = methyl.

Compound **2** was isolated as greenish brown oil; $[\alpha]^{25}D$ - 91º (c 0.1, $CHCl_3$)and exhibited IR absorption bonds at 1738 and 1727 cm^{-1}, its molecular formula was assigned as $C_{33}H_{41}O_9$ from positive HRFABMS spectrum at m/z 582.3086. The ^1H and ^{13}C NMR ($CDCl_3$, Table-2) spectra of compound **2** exhibited signals for one benzoate group, H-3',7' appeared as double doublet at δ_H 8.09 (2H, *J* = 7.1, 1.4 Hz, δ_C 129.6), H-4',6' at δ_H 7.47 (2H, t, *J* = 7.1 Hz, δ_C 128.4) and H-5' at δ_H 7.60 (1H, tt, *J* = 7.1, 1.4 Hz, δ_C133.2) and the carbonyl carbon appeared at δ_C 165.1. Additionally, three acetate groups were detected at δ_H 1.55 (δ_C 169.9, 20.7), 2.19 (δ_C 170.3, 21.1) and 1.95 (δ_C 169.1, 20.3). The structure of the main skeleton was

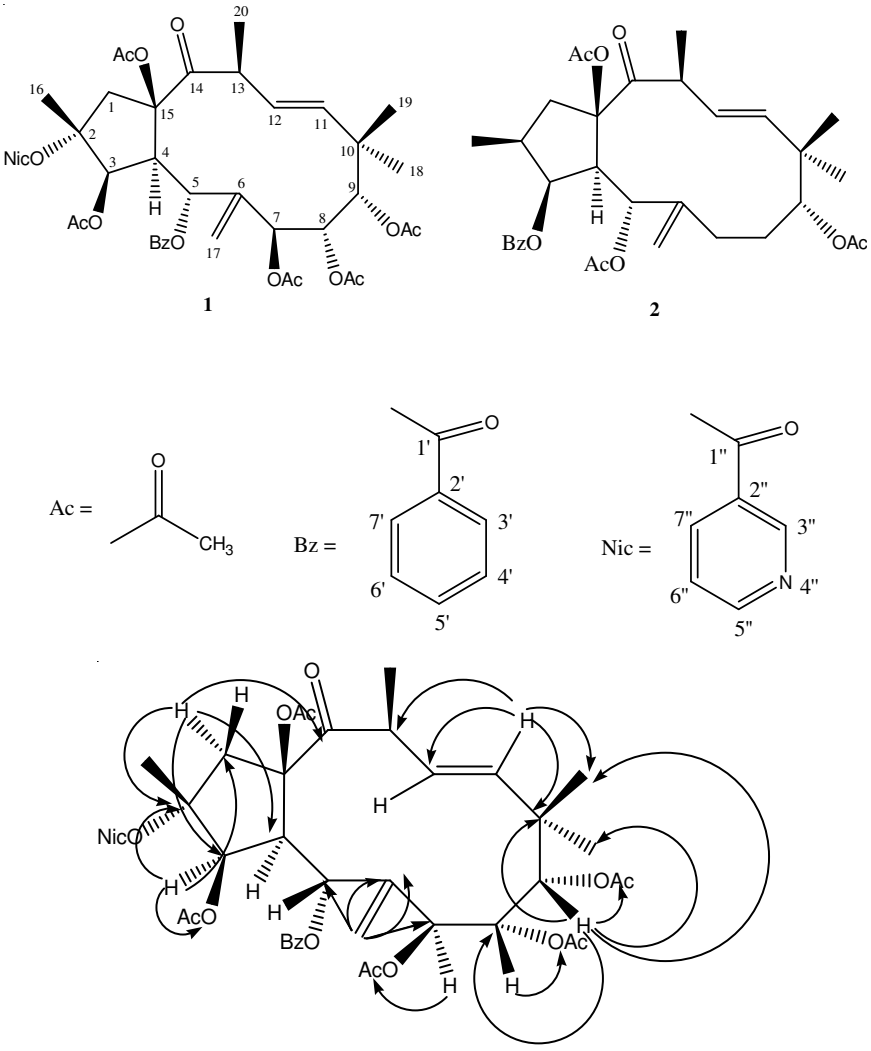

Fig. 1. Selected HMBC correlations of compound **1**

shown to be diterpene from the presence of 20 carbons in the ^{13}C NMR and DEPT experiments. Comparison of the 1D and 2D spectral data of **1** with those of **2** revealed the presence of *trans*-CH_{11}=CH_{12}-$CH_{13}(CH_3)$- moiety, H-11 appeared at δ_H 6.03 (1H, d, J = 15.9 Hz), H-12 at δ_H 5.43 (1H, dd, J = 15.9, 9.6 Hz) and H-13 at δ_H 3.59 (1H, dd, J = 9.6, 6.3 Hz). Also, its ^1H NMR and ^1H-^1H COSY spectra proved the presence of -CH_2-CH_2- moiety, -CH(O)-CH-CH(O)-$CH(CH_3)$-CH_2- moiety and exomethylene protons at δ_H 5.00 (br s) 5.08 (br s). Two tertiary methyl groups were detected at δ_H 1.19 and 1.18, two secondary methyl group were found at δ_H 0.96 and 1.17. Furthermore, ^{13}C NMR and DEPT of 2 indicated the presence of one

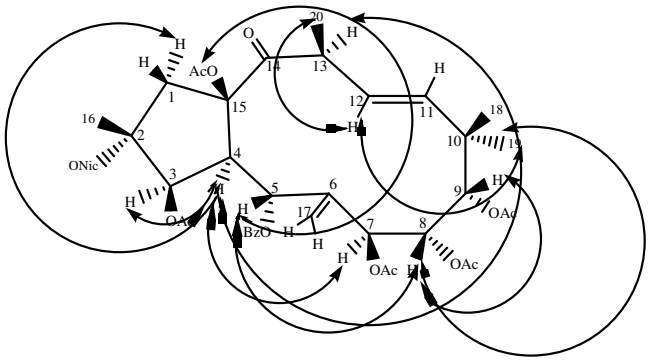

Fig. 2. Selected NOE correlations of compound **1**

TABLE-2
NMR SPECTRAL DATA OF COMPOUND **2*** (500 MHz, $CDCl_3$, 25 °C, δ-VALUES)

Protons	δ_H (*J* in Hz)**	Carbons*	HMBC	NOESY
1_β	1.80 (dd, 14.3, 12.9)	46.5 (t)		H-16
1_α	3.08 (dd, 14.3, 7.4)		C-2, C-3, C-4, C-14, C-15	H-2,
2_α	2.25 (m)	38.9 (d)		H-1
3_α	5.79 (t, 3.3)	76.9 (d)	C-1´, C-1, C-15	H-4, H-16, H-17_b
4_α	2.17 (m)	53.9 (d)	C-6, C-14	H-3, H-5, H-11, H-17_b
5_β	5.80 (br s)	68.7 (d)	C-3, C-4, C-6, C-17, 5-OAc	H-4, H-7_β, H-8_β, H-13, H-17_b
6		144.9 (s)		
7_α	2.36 (ddd, 13.7, 9.3, 9.3)	30.0 (t)	C-5, C-6, C-8, C-17	H-17_b
7_β	1.81 (m)			H-5
8_α	2.20 (m)	37.9 (t)		
8_β	2.87 (m)		C-6, C-7, C-9	H-5, H-11
9_β	5.21 (m)	81.1 (d)	C-7, C-8, C-10, C-11, C-18, C-19, C=O (9-OAc)	H-8, H-18, H-19
10		49.5 (s)		
11	6.03 (d, 15.9)	137.4 (d)	C-10, C-13, C-18, C-19	H-4, H-5, H-8, H-13
12	5.34 (dd, 15.9, 9.6)	132.2 (d)	C-10, C-13, C-20	H-20
13_α	3.59 (dd, 9.1, 6.3)	44.7 (d)	C-11, C-12, C-14, C-20	H-5, H-11
14		213.4 (s)		
15		92.6 (s)		

Protons	δ_H (*J* in Hz)**	Carbons*	HMBC	NOESY
16	0.96 (d, 6.6)	13.4 (q)	C-1, C-2, C-3	H-1_β, H-3
17_a	5.00 (br s)	114.1 (t)	C-6, C-7	H-7_α
17_b	5.08 (br s)		C-5, C-6	H-3, H-4, H-5
18	1.18 (s)	25.9 (q)	C-9, C-10, C-11, C-19	
19	1.19 (s)	22.6 (q)	C-9, C-10, C-11, C-18	
20	1.17 (d, 6.3)	19.8 (q)	C-12, C-13, C-14	H-12
1´		165.1 (s)		
2´		130.6 (s)		
3´, 7´	8.09 (dd, 7.1, 1.4)	129.6 (d)	C-1´, C-5´	5-OAc, 15-OAc
4´, 6´	7.47 (t, 7.1)	128.4 (d)	C-2´	
5´	7.60 (tt, 7.1, 1.4)	133.2 (d)	C-3´, C-7´	
5-OAc	1.55 (s)	169.9 (s), 20.7 (q)	C=O (5-OAc)	H-3´, H-7´
9-OAc	1.95 (s)	169.1 (s), 20.3 (q)	C=O (9-OAc)	H-18
15-OAc	2.19 (s)	170.3 (s), 21.1 (q)	C=O (15-OAc)	H-3´, H-7´

*Multiplicity was determined by DEPT experiments
**Assignments by 1D and 2D spectral data.
***s = quaternary, d = methine, t = methylene, q = methyl.

ketone δ_C 213.4. The connectivity of these partial structures and the positions of the acyl groups were determined from HMBC (Table-2). In this experiment, C-H correlations were observed between the protons of the exomethylene group at δ_H 5.00 and 5.08 (H-17_a and H-17_b) and the carbon signal at δ_C 144.9 (C-6), 30.0 (C-7) and 68.7 (C-5), between the proton signal at δ_H 2.87 (H-8_b) and the carbon signal at δ_C 144.9 (C-6), 30.0 (C-7) and δ_C 81.8 (C-9). The placement of the benzoate group at C-3 was suggested from the correlation between H-3 (δ_H 5.79) and the carbonyl carbon at δ_C 165.1. The remaining three acetate groups must be at C-5, C-15 and C-9, where H-5 (δ_H 5.80) exhibited correlation with the carbonyl carbon at δ_C 169.9. The unusual high field chemical shift of the acetate group at C-5 (δ_H 1.55) due to the anisotropic shielding effect of the benzoate moiety at C-3. The relative configuration of compound **2** was investigated by means of NOESY measurements. The observed NOE correlations between H3/H-17b, H-4/H-11, H-4/H17b, H-7_α/H-17a and H-11/H-13 supported the α-orientation of all protons. The NOE correlations between H-1_β/H-16, H-5/H-7_β, H-5/H-8_β, 15-OAc/H-20, 9-OAc/H-18, 15-OAc/H-3',7' indicated the β-orientation of those protons.

Diterpenoids containing the same skeleton as **1** and **2**, jatrophane skeleton, are rare in the plant kingdom and their occurrence is limited to

few species of the genus *Euphorbia*. Guyonianin C appears to be rare jatrophane containing two phenyl moieties from the genus *Euphorbia*.

Antibacterial screening

In vitro, screening experiments for antibacterial activities of guyonianin C and D was subjected to biological testing . To substantiate the antibacterial results, we screened guyonianin C and D against an assortment of two Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Serratia* Sp., *Pseudomonos* Sp.) using ampicillin and amoxillin as a reference standard. The minimum inhibitory concentrations (MICs, μg/mL) were determined using standard agar dilution method[16]. The MIC value is summarized in Table-3.

TABLE-3
ANTIMICROBIAL ACTIVITIES OF GUYONIANIN C AND D
(DRY DMSO AS SOLVENT)

Test Organism	Guyonianin C[c]	Guyonianin D[c]	Ampicillin[d]	Amoxillin[d]
Gram +ve Strain				
B. cereus	11[a]	No effect[a]	10[a]	No effect[a]
	19[b]	No effect[b]		
S. aureus	No effect[a]	No effect[a]	8[a]	No effect[a]
	6[b]	No effect[b]		
Gram -ve Strain				
Serratia sp.	No effect[a]	No effect[a]	11[a]	13[a]
	No effect[b]	No effect[b]		
Pseudomonas sp.	No effect[a]	No effect[a]	11[a]	13[a]
	No effect[b]	No effect[b]		
E. coli	No effect[a]	No effect[a]	11[a]	

[a]Values show the zone of inhibition in mm; conc. of the samples was 200 μg/mL
[b]Values show the zone of inhibition in mm; conc. of the samples was 400μg/mL
[c]Data are the mean of five measurements with neglected standard errors.
[d]Reference antibiotics were carried out at 200 μg/mL only.

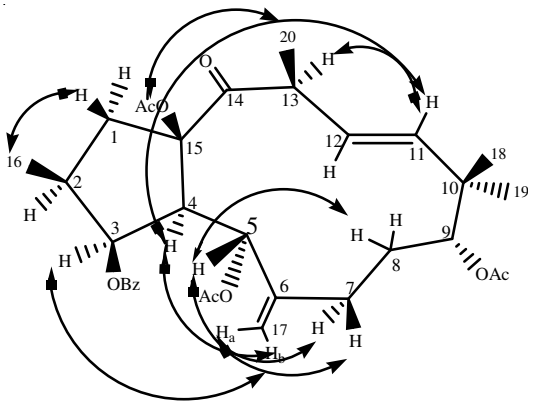

Fig. 3. Selected NOE correlations of compound **2**

From the obtained data, it is clear that guyonianin C posses higher activity against Gram-positive strain, particulerly *Bacillus cereus*. On the contrary, Gram-negative strains not affected at tested concentrations as shown in Table-3. Our results are in agreement with those reported earlier by Joklik *et al.*[17], they reported that some antibiotics such as ampicillin and amoxillin have been developed as inhibitors of cell wall synthesis of bacterial cell . So, guyonianin C has the common structural feature of penicillins exhibit antibacterial activities .

REFERENCES

1. T. Morgenstern, M. Bittner, M. Silva, P. Aqueveque and J. Jakupovic, *Phytochemistry*, **41**, 1149 (1996).
2. F. Jeske, J. Jakupovic and W. Berendsohn, *Phytochemistry*, **40**, 1743 (1995).
3. G. Appendino, P. Spagliardi, M. Ballero and G. Seu, *Fitoterapia*, **73**, 476 (2002).
4. G. Corea, E. Fattorusso, V. Lanzotti, O. Tagliata-Scafati, G. Appendino, M. Ballero, P.N. Simon, C. Dumontet and A. Di Pietro, *J. Med. Chem.*, **46**, 3395 (2003).
5. S.A.M. Abdelgaleil, S.M.I. Kassem, M. Doe, M. Baba and M. Nakatani, *Phytochemistry*, **58**, 1135 (2001).
6. J. Hohmann, D. Rédei, F. Evanics, A. Kálmán, G. Argay and T. Bartok, *Tetrahedron*, **56**, 3619 (2000).
7. T.X. Zhou, G.H. Bao, Q.G. Ma, G.W. Qin, C.T. Che, Y. Lv, C. Wang and Q.T. Zheng, *Tetrahedron Lett.*, **44**, 135 (2003).
8. J. Hohmann, F. Evanics, G. Dombi and P. Szabó, *Tetrahedron Lett.*, **42**, 6581 (2001).
9. M. Ilyas, M. Parveen and K.M.Y. Amin, *Phytochemistry*, **48**, 561 (1998).
10. E.M.C. Lima, J.M.R. Medeiros and L.B. Davin, *Phytochemistry*, **63**, 421 (2003).
11. J.G. Shi, Y.P. Shi and Z.J. Jian, *Phytochemistry*, **45**, 343 (1997).
12. A.F.M. Rizk, A.F.M. Youssef, M.A. Diab and H.M. Salem, *Pharmazie*, **31**, 405 (1976).
13. J. Jakupovic, F. Jeske, T. Morgenstern, F. Tsichritzis, J.A. Marco and W. Berendsohn, *Phytochemistry*, **47**, 1583 (1998).
14. G. Appendino, S. Jakupovic, G.C. Tron, J. Jakupovic, V. Milon and M. Ballerom, *J. Nat. Prod.*, **61**, 749 (1998).
15. G. Corea, C. Fattorusso, E. Fattorusso and V. Lanzotti, *Tetrahedron*, **61**, 4485 (2005).
16. F. Steers, F.L. Foltz and B.S. Graves, *Antibiot. Chemother.*, **9**, 305 (1959).
17. W.K. Joklik, H.P. Willet, D.B. Amos and C.M. Wifert, Zinsser Microbiology, Norwalk, CT: Appleton and Lange edn. 20, p. 155 (1992).

(*Received*: 23 June 2006; *Accepted*: 21 April 2007) AJC-5592