Tetrahedron Vol 49, No. 1, pp. 141-150, 1993
Printed in Great Britain

0040-4020/93 $5.00+.00

Gibepyrones: α-Pyrones from *Gibberella fujikuroi*

Alejandro F. Barrero*, Juan E. Oltra, María M. Herrador, Eduardo Cabrera, Juan F. Sanchez, José F. Quílez, Francisco J. Rojas, José F. Reyes.

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Granada, 18071. Granada. Spain.

(*Received in UK* 31 *July* 1992)

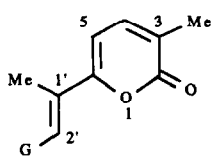
Abstract: Six α-pyrones, called gibepyrones A-F, were identified in three different culture media of *G. fujikuroi* (IMI 58289). Their structures were determined using both spectroscopic techniques and chemical synthesis. Their antimicrobial activity has been screened.

INTRODUCTION

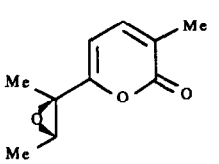
Apart from many diterpenoids (*ent*-kaurenoids and gibberellins)[1], *G. fujikuroi* produces a noticeable diversity of substances, such as fusaric acid derivatives[2], N-jasmonoylisoleucine[3], O-demethylanhydrofusarubin[4], bikaverin[5], cyclonerodiol[6], moniliformin[7], steroids[8], carotenoids[9], fusarin C[10] and fumonisin B-1[11]. No metabolites with an α-pyrone related structure have so far been reported, however we have identified six novel isoprenoid α-pyrones, gibepyrones A-F (**1-4**, **6** and **7**) in three different culture media of the wild strain IMI 58289. This paper describes their structural characterization, chemical synthesis and a screening of their antimicrobial activity.

METHODS AND RESULTS

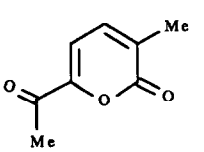
Gibepyrones A, B, and D (**1**, **2**, and **4**) were isolated from an ICI 10% culture medium[12], in which glucose had been substituted by calcium lactate (96.88 g/l) as carbon source (Assay I).



1 G= CH_3, **2** G= CH_2OH, **3** G= CHO,
4 G= CO_2H, **5** G= CO_2Me



6



7

Gibepyrone A has the molecular formula $C_{10}H_{12}O_2$, as deduced from its mass (M+ at m/z:164) and ^{13}C NMR spectra (Table 1). Its IR (strong absortion at 1716 cm^{-1}) and UV (λ_{max}=241 and 336 nm) spectra are in accordance with an extended α-pyrone chromophore[13]. The ^1H NMR spectrum shows two signals, at 6.01 (d,

J=7.0 Hz) and 7.07 ppm (dq, J=7.0 Hz, J=1.3 Hz), due to two vicinal protons in a 3,6 disubstituted α-pyrone system. The most deshielded hydrogen, H-4, is in ß position to the carbonyl group and shows a long range coupling with the methyl that appears at 2.03 ppm (d, J=1.3 Hz, $C\underline{H}_3$-C-3). An 1'-methylpropenyl residue, located on C-6, bears the methyls at 1.77 ppm (br d, J=7 Hz, H-3') and 1.79 ppm (br s, $\underline{C}H_3$-C-1') and the olefinic proton at 6.51 ppm (qq, J=7.0 Hz, J=1.0 Hz, H-2'). The above-mentioned connectivities were confirmed by a 2D NMR $^1H/^1H$ homonuclear correlation (Table 2). The ^{13}C NMR spectrum also agrees with a skeleton of 3-methyl-6-(1'-methylpropenyl)-α-pyrone, and $^1H/^{13}C$ direct and long range correlation experiments (Table 3) were performed in order to assign its signals unambiguously, especially that of $\underline{C}H_3$-C-1'. This carbon appears at high field (12.0 ppm) due to the shielding effect of methyl C-3' in γ-*syn* disposition[14], showing the 1'*E* configuration of **1**. A related compound, fusalanipyrone, isolated from *Fusarium solani*[15], has been described as the 1'Z isomer of **1**.

Table 1. ^{13}C NMR Data of Compounds **1**, **2**, **5**-**8** and **11**-**16**.

	1	**2**	**5**	**6**	**7**	**8**	**11**	**12**	**13**	**14**	**15**	**16**
C-2	163.5	163.2	162.2	163.2*	161.4	163.9*			164.4*	164.4*	164.3*	164.4*
C-3	122.9	128.3	127.4	124.1	132.1	122.7			123.1	123.1	122.8	122.8
C-4	139.9	139.7	138.8	139.5	138.0	139.7	140.1	139.6	140.3	140.2	139.7	139.6
C-5	100.4	101.9	105.4	100.8	107.2	101.5	101.9	105.7	104.0	103.5	103.6	103.1
C-6	159.8	160.0	157.5	162.5*	153.4	165.3*			164.5*	164.5*	163.9*	163.8*
C-1'	126.8	124.5	142.5	57.6	191.5	41.5	132.7	132.7	41.3	40.9	41.6	41.0
C-2'	128.0	131.1	118.8	61.5	25.9	137.9	132.0	132.4	73.5	73.3	72.6	71.9
C-3'	14.1	59.6	167.0	14.1		116.1	14.0	15.6	64.2	64.7	65.0	65.2
$\underline{C}H_3$-C-3	16.6	16.8	17.0	17.6	17.3	16.4	16.8	16.7	16.6	16.6	16.5	16.5
$\underline{C}H_3$-C-1'	12.0	12.7	13.5	13.9		17.5	56.8	65.6	15.0	13.6	14.8	13.2
$CO_2\underline{C}H_3$			18.2									

Assignments of **1** have been achieved on the basis of 2D NMR experiments (Table 3). Those of the rest of compounds were realized by their DEPT spectra, tabular data[14] and their relation with **1**. Signals corresponding to triphenylmethyl group: for **15** δ=86.7 (C-1"), 127.1 (C-3"), 128.7 (C-4"), 129.0 (C-5"), 143.6 (C-2") and for **16** δ=82.0 (C-1"), 127.2 (C-3"), 127.9 (C-5"), 128.7 (C-4"), 143.6 (C-2"). *Tentative assignments.

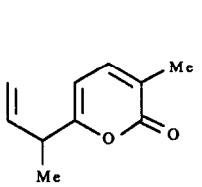
Table 2. $^1H/^1H$ Correlations (COSY) of **1**

H (δ)		Correlated Hydrogen
H-4	(7.07)	H-5, $C\underline{H}_3$-C-3
H-5	(6.01)	H-4, $C\underline{H}_3$-C-3, $C\underline{H}_3$-C-1'
H-2'	(6.51)	H-3', $C\underline{H}_3$-C-1'
H-3'	(1.77)	H-2'
$C\underline{H}_3$-C-3	(2.03)	H-4, H-5
$C\underline{H}_3$-C-1'	(1.79)	H-5, H-2'

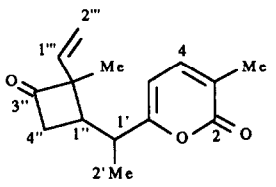
Table 3 $^{1}H/^{13}C$ Direct and Long Range (J=9 Hz) Correlations of **1**

H (δ)		Direct (δ)	Long Range (δ)
H-4	(7.07)	C-4 (139.9)	
H-5	(6.01)	C-5 (100.4)	
H-2'	(6.51)	C-2' (128.0)	
H-3'	(1.77)	C-3' (14.1)	
$C\underline{H}_3$-C-3	(2.03)	$\underline{C}H_3$-C-3 (16.6)	C-2 (163.5), C-3 (122.9), C-4 (139.9)
$C\underline{H}_3$-C-1'	(1.79)	$\underline{C}H_3$-C-1' (12.0)	C-6 (159.8), C-1' (126.8)

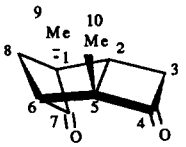
Gibepyrone A was synthesized following the procedure of Rey *et al.*[16] (reaction time was reduced from 30 to 2 hours), by treating tigloyl chloride with triethylamine. Besides α–pyrones **1** (35%) and **8**[16] (35%) we obtained two new minor compounds, **9** (2.5%) and **10** (6.5%). The structure of pyrone **9** was assigned by comparing its spectroscopic data with those of **8** whereas the structural characterization of **10** was completed using nOe and two dimensional NMR experiments: COLOC, J=10 Hz, and long range COSY, J=1Hz (*cf.* Experimental section).The presence of **9** and **10** in the reaction medium can be explained in terms of the methylvinylketene formed by the 1,4-eliminitation of HCl from tigloyl chloride. Cyclobutanone **9** would be obtained by 2+2 cycloaddition between **8** and methylvinylketene. The formation of **10** would involve 2+2 cycloaddition between two molecules of methylvinylketene, followed by a second intramolecular cycloaddition.



8

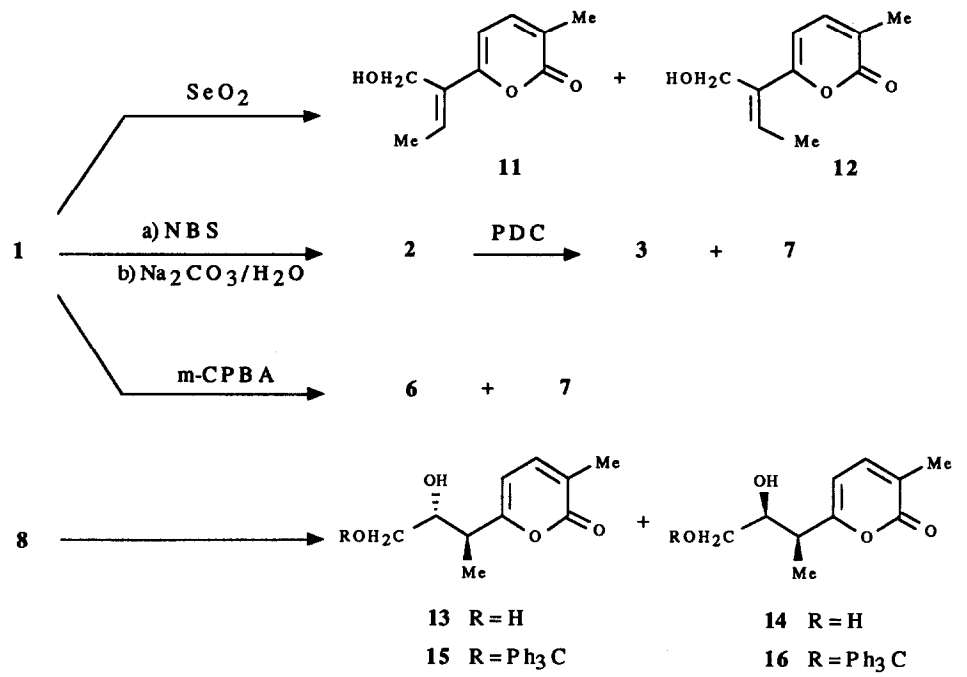


9



10

Gibepyrone B (**2**) is the 3'-hydroxyderivative of **1**. It displays the molecular formula $C_{10}H_{12}O_2$, inferred from its mass and ^{13}C NMR spectra (Table 1). Its IR spectrum shows an absorbtion band at 3414 cm^{-1} (OH), and in its ^{1}H NMR spectrum, which is very similar to that of **1**, a doublet appears at 4.38 ppm (J=6.6 Hz, $C\underline{H}_2OH$) and only two methyl signals, $C\underline{H}_3$-C-1' (1.88 ppm) and $C\underline{H}_3$-C-3 (2.10 ppm). The ^{13}C NMR shows the signal corresponding to $\underline{C}H_3$-C-1' at δ 12.7 ppm, indicating 1'*E* stereochemistry. NOe-dif experiments (Table 4) confirmed this configuration and revealed the preferred s-*trans* conformation of the C-6-C-1' bond. The chemical synthesis of **2** was first attempted by oxidation of **1** with SeO_2, resulting in the hydroxylation at $\underline{C}$-C-1' and yielding a mixture of the isomers **11** and **12**. Alternatively, we tried to synthesize **2** from glycols **13** and/or **14**, and then selectively dehydrating the secondary hydroxyl. The reaction of **8** with OsO_4 generated a mixture of isomers **13** and **14**. Treatment of these compounds with trityl chloride led to the selective protection of the primary hydroxyl, giving the diastereomers **15** and **16**. Atempts to dehydrate the secondary hydroxyl with $POCl_3$ were unsuccessful. Other attempts to protect-dehydrate **11** and **12** regioselectively by means of their mesylated or tosylated derivatives also failed, and resulted in the decomposition of the α-pyrone ring. Finally, gybepyrone B



was prepared by the reaction of **1** with NBS and subsequent treatment of the bromo derivative with Na_2CO_3 in aqueous acetone.

Gibepyrone D (**4**), isolated as methyl ester (**5**), was characterized by comparing its spectroscopic properties with those of **1** and **2**. Its 1'*E* configuration was established on the basis of the chemical shift of $\underline{C}H_3$-C-1' (Table1).

Table 4. NOe-dif experiences in **2**, **11** and **12**

2		**11**		**12**	
Irrad. H	NOes	Irrad. H	NOes	Irrad. H	NOes
H-2'	H-3'	$C\underline{H}_2OH$	H-3', H-5	$C\underline{H}_2OH$	H-2'
H-3'	H-2', $C\underline{H}_3$-C-1'				
$C\underline{H}_3$-C-1'	H-3', H-5				

When the concentration of calcium lactate was reduced by half (Assay II), apart from **1** and **2**, gibepyrones E and F (**6**,**7**) were isolated. The mass spectrum of **6** shows the molecular peak at m/z 180, in accordance with a molecular formula of $C_{10}H_{12}O_3$. Its ^1H NMR spectrum displays a quadruplet at 3.10 ppm (H-2'), assignable to a proton of oxirane ring. In the ^{13}C NMR spectrum (Table I) $\underline{C}H_3$-C-1' appears at 13.9 ppm, indicating its *syn* disposition with respect to C-3'. Gibepyrone E has optical activity.

The IR spectrum of gibepyrone F (**7**) presents two absorption bands, characteristic of carbonyl groups at

1701 cm-1 (α-pyrone) and 1689 (conjugated ketone). The ^{1}H NMR shows the presence of two methyl groups at 2.18 ($C\underline{H}_3$-C-3) and 2.50 ppm ($C\underline{H}_3$-CO-), and signals corresponding to the hydrogens H-4 and H-5 of the 3,6 disubstituted α-pyrone system appear at 7.24 and 6.95 ppm respectively. The oxidation of **1** with m-CPBA generated a mixture of epoxide **6** and ketone **7**. 4-Methoxyderivative of **7**, vermopyrone, has recently been isolated from *Gliocladium vermoesenii*[17].

Despite following the isoprenic rule, gibepyrone A is structurally related to fusalanipyrone, which suggests that the former compound may derive from a tetraketide, which would later undergo two methylations[18]. Starting from **1**, the existence of hydroxylating enzymes in *G. fujikuroi* accounts for the biosynthesis of **2**, which after subsequent oxidation pathways would produce **4**. Gibepyrone E is derived from the epoxidation of the exocyclic double bond of **1**, and gibepyrone F resulting from oxidative cleavage of the same bond.

The coexistence of **2** and **4** in the culture medium pointed to the possibility of a biogenetic intermediate with an aldehyde function at C-3'. Not being able to isolate this product in any broth culture we tried to determine its occurrence by means of HPLC analysis of the media. Accordingly, we synthesized **3** for use as standard. The oxidation of **2** with PDC [19] generated **3** as well as ketone **7**. Even though aldehyde **3** (gibepyrone C) had not been isolated, it was detected in all the media analyzed (*cf.* Table 5).

Finally, we carried out a culture in ICI 10% medium[12] under conditions suitable for the production of GA_3[20] (Assay III). The presence of neutral gibepyrones in the media of assays I, II and III was detected by HPLC-UV, using authentic samples as standard. The results are shown in Table 5.

The antimicrobial activity of the gibepyrones has been tested against a group of microorganisms (*cf.*: Experimental section). Gibepyrone A and B were moderately active, the former preventing the growth of *Bacillus subtilis* (MIC=100 μg/ml), *Staphylococcus aureus* (200 μg/ml), *Saccharomyces cerevisiae* (100 μg/ml) and *Candida albicans* (200 μg/ml); gibepyrone B was active against *S. aureus* (200 μg/ml) and *S. cerevisiae* (100 μg/ml).

Table 5. Neutral Gibepyrones in the Media of Assays I, II, III

	1	**2**	**3**	**6**	**7**
Rt (min)	11.03	3.47	4.38	5.70	3.91
UV, λ max (nm)	241.336	240.332	256.347	228.303	250.314
Assay I	+	+	+	+	+
Assay II	+	+	+	+	+
Assay III	+	+	+	-	+

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer 983 G spectrometer with samples between sodium chloride plates or as potassium bromide pellets. ^{1}H NMR spectra were recorded on Bruker WP 80 SY (80 MHz) and Bruker AM 300 (300 MHz) spectrometers using TMS or $CHCl_3$ as standard and $CDCl_3$ as solvent. ^{13}C NMR spectra were run at 75 MHz on a Bruker AM 300 instrument. 2D NMR (^{1}H/^{1}H and ^{1}H/^{13}C correlations) experiments were performed on a Bruker AM 300 spectrometer. Chromatographic separations were carried out in

a conventional column on Merck silica gel 60 (70-230 mesh) using eluents of increasing polarity from hexane to diethyl ether, except when the contrary is indicated. High-pressure liquid chromatography (HPLC) was performed with a 500 B Konic instrument equipped with a linear UVIS-206 multiple wave-length detector, using a Spherisorb S5 ODS2 250x4.6 mm column, an isocratic mixture of CH_3CN:H_2O/5% CH_3CN 4:6 and a column-flow-rate of 1ml/min.

Culture conditions

Gibberella fujikuroi, strain IMI 58289, was obtained from the Commonwealth Mycological Institute, Kew, Surrey, U.K. The liquid medium was inoculated with spores and incubated in darkness at 30^0 C, changing the stirring rate, carbon source, medium volume and incubation time. Three assays were carried out: I) 35 days incubation in 4l of 10% ICI medium[12], the glucose being replaced by 96.88 g/l of calcium lactate, in 6l flasks with a stirring rate of 120 rpm. II) 35 days incubation in 4l of 10% ICI medium, the glucose being replaced by 48.44 g/l of calcium lactate, in 6l flasks with a stirring rate of 120 rpm. III) 14 days incubation in 125 ml of 10% ICI medium in 250 ml flasks with a stirring rate of 250 rpm.

Extraction and purification

After incubation the culture media were filtered and extracted with EtOAc at neutral pH. The neutral extracts of the assays I and II were chromatographed over silica gel. Compounds **1** and **2** were isolated from the extract of assay I and substances **1**, **2**, **6** and **7** from assay II. All the neutral extracts were analyzed by HPLC (*cf.* Table 5). The acid extract from assay I was methylated with CH_2N_2 and subsequently chromatographed over silica gel. Methyl ester **5** was isolated.

Gibepyrone A (**1**). Eluted with hexane- Et_2O 85:15; concentration in the culture medium (assays I and II) 10 μg/ml; oil: IR (neat): ν 2962, 2924, 2854, 1716 (C=O), 1643 (C=C), 1560, 1460, 1390, 1260, 1110, 1030, 800, 770 cm^{-1}; ^{1}H NMR (300 MHz): δ 1.77 (br d, J=7 Hz, H-3'), 1.79 (br s, C$\underline{H}_3$-C-1'), 2.03 (d, J=1.3 Hz,C$\underline{H}_3$-C-3), 6.01 (d, J=7 Hz. H-5), 6.51 (qq, J=7 Hz, J=1 Hz, H-2'), 7.07 (dq, J=7.0 Hz, J=1.3 Hz, H-4); ^{13}C NMR in Table 1; EIMS m/z (rel. int.): 164 [M]$^+$ (68), 149 [M-CH_3]$^+$ (1), 136 [M-CO]$^+$ (57), 135 (14), 121 (100), 109 [$C_6H_5O_2$]$^+$ (16), 93 (29), 91 (22), 81 (6), 77 (17), 55 (21), 53 [C_4H_5]$^+$ (77). Synthesis: we followed the procedure of Rey *et al*[16], modified as follows: a chloroform solution of Et_3N (1.79 g) was added to a solution of tygloyl chloride (2g) in chlroform (16 ml) at 0^{0}C under inert atmosphere. The mixture was stirred at room temperature for 2 h. The solvent was concentrated *in vacuo* and the resulting residue extracted with ether. Evaporation of the ether gave a mixture which was chromatographed on a silica gel column. **1** (yield 35%), **8** (35%) and minor compounds **9** (2.5%) and **10** (6.5%) were isolated.

3-methyl-6-(1-methylallyl)-α-pyrone (**8**)[16]. Eluted with hexane-Et_2O, 88:12; ^{13}C NMR in Table 1; EIMS (rel.int): 164 [M]$^+$ (21), 149 [M-CH_3]$^+$ (16), 136 [M-CO]$^+$ (16), 135 [M-CHO]$^+$ (2), 121 (18), 109 [$C_6H_5O_2$]$^+$ (71), 93 (6), 91 (10), 81 (12), 77 (8), 55 (8), 53 [C_4H_5]$^+$ (100).

3-methyl-6-[1'-(2''-methyl-3''-oxo-2''-vinylcyclobutyl)ethyl]-α-pyrone (**9**). Eluted with hexane-Et_2O 25:75 ; oil ; IR (neat): ν 3087, 2970, 2927, 1778 (C=O), 1714 (C=O), 1640 (C=C), 1580, 1452, 1382, 1040, 996, 924 cm^{-1}; ^{1}H NMR (300 MHz): δ 1.26 (d, J=6.1 Hz, H-2'), 1.31 (s, C$\underline{H}_3$-C-2''), 2.06 (d, J=1.0Hz, C$\underline{H}_3$-

C-3), 2.67 (m, H-1' and H1''), 2.83 (m, H-4''), 5.14 (d, J=10.6 Hz, H-2'''a), 5.17 (d, J=17.4 Hz, H-2'''b), 5.88 (dd, J=10.6 Hz, J=17.4 Hz, H-1'''), 5.92 (d, J=6.5 Hz, H-5), 7.06 (dq, J=6.5 Hz, J=1.0 Hz, H-4); ^{13}C NMR (75 MHz): δ 163.9* (C-2), 123.5 (C-3), 139.6 (C-4), 102.5 (C-5), 164.8* (C-6), 38.4** (C-1'), 18.8 (C-2'), 40.1** (C-1''), 66.9 (C-2''), 209.2 (C-3''), 47.0 (C-4''), 138.5 (C-1'''), 114.9 (C-2'''), 16.6 ($\underline{C}H_3$-C-3), 15.6 ($\underline{C}H_3$-C-2''), *, **: interchangeable values; EIMS (rel. int.): 246 [M]+ (1), 218 [M-CO]+, 204 (2), 189 (5), 161 (8), 138 (44), 109 $[C_6H_5O_2]^+$ (77), 81 (32), 53 $[C_4H_5]^+$ (100).

(1RS, 2RS, 5RS, 6SR)-1,5-dimethyl-4,7-dioxotricycle-[4,1,1,0^{2,5}]-octane (**10**). Eluted with hexane-Et_2O 8:2; oil; IR (neat) ν max: 2961, 2925, 2868, 1778 (C=O), 1449, 1385 cm^{-1}. ^1H NMR (300 MHz): δ 1.27 (s, H-9), 1.33 (s, H-10), and two ABX systems, the first appearing at 1.65 (dd, A of ABX, J_{AB}=7.8 Hz, J_{AX}=2.3 Hz, H-8α), 1.86 (d, B of ABX, J_{AB}=7.8 Hz, H-8β), 2.82 (d, X of ABX, J_{AX}=2.3 Hz, H-6), and the second at 2.43 (dd, A of ABX, J_{AB}=3.2 Hz, J_{AX}=7.6 Hz, H-2), 2.61 (dd, B of ABX, J_{AB}=3.2 Hz, J_{BX}=18.8 Hz, H-3α), 3.01 (dd, X of ABX, J_{AX}=7.6 Hz, J_{BX}=18.8 Hz, H-3β). ^{13}C NMR (75 MHz):δ 68.5 (C-1), 39.3 (C-2), 45.9 (C-3), 209.5 (C-4), 70.9 (C-5), 59.9 (C-6), 197.2 (C-7), 33.7 (C-8), 10.1 (C-9), 15.7 (C-10); COLOC correlations (C/H): C-1/H-9; C-2/H-9 and H-10; C-4/H-10 and H-3β; C-5/H-10; C-6/H-10; C-7/H-9; C-9/H-8α and H-8β; long range COSY correlations (H/H): H-2/H-6, H-8β, H-9 and H-10; H-3α/H-8α; H-3β/H–8α; H-6/H-2 and H-10; H-8α/H-2, H-3α, H–3β and H-9; H-8β/H-9; H-9/H-2, H-8α and H-8β; H-10/H-2 and H6; EIMS m/z (rel. int.): 164 [M]+ (14), 136 [M-CO]+ (3), 83 $[C_5H_7O]^+$ (100), 55 (76).

Gibepyrone B (**2**). Eluted with hexane-Et_2O 1:9; concentration in the culture medium of the assay I, 5μg/ml; assay II 3μg/ml; oil; IR (neat): ν 3414 (OH), 2927, 1705 (C=O), 1640 (C=C), 1570, 1377,1250, 1122, 1041, 770 cm^{-1}; ^1H NMR (300 MHz): δ 1.88 (d, J=1 Hz, $C\underline{H}_3$-C-1'), 2.10 (d, J=1.2 Hz, $C\underline{H}_3$-C-3), 4.38 (d, J=6.6Hz, H-3'), 6.14 (d, J=6.9 Hz, H-5), 6.63 (tq, J=6.6 Hz, J=1 Hz, H-2'), 7.12 (dq, J=6.9 Hz, J=1.2 Hz, H-4); ^{13}C NMR in Table I; EIMS (rel. int.): 180 [M]+ (17), 152 [M-CO]+ (10), 151 [M-CHO]+ (100), 123 (5), 109 $[C_6H_5O_2]^+$ (25), 93 (11), 91 (13), 81 (10), 77 (9), 53 $[C_4H_5]^+$ (43). Preparation: NBS (108 mg) and a catalytic amount of benzoyl peroxide were added to a solution of **1** (100 m/g) in CH_2Cl_2 (15 ml). The mixture was refluxed for 24 h. Removal of the precipitated succinimide and solvent gave the crude product which was dissolved in a 50% acetone-water solution (15ml). Na_2CO_3 was added under stirring. The resulting solution was stirred for a further 24 h and then extracted with EtAcO. Evaporation of the solvent gave a residue, which after chromatography in a silica gel column yielded 12 mg of **2**.

(1'E)-3-methyl-6-(1'-methyl-2'-methoxycarbonylvinyl)-α-pyrone (**5**). Eluted with hexane-Et_2O 65:35; corresponds to 4 μg/ml of gibepyrone D in the medium; oil; IR (neat): ν 2959, 2928, 2871, 1710 (C=O), 1612, 1562, 1512, 1454, 1435, 1262, 1172, 1118, 909, 797 cm^{-1}; ^1H NMR (300 MHz): δ 2.12 (d, J=1.3 Hz, $C\underline{H}_3$-C-3), 2.35 (d, J=1 Hz, $C\underline{H}_3$-C-1'), 3.74 (s, OMe), 6.42 (d, J=7 Hz, H-5), 6.70 (q, J=1 Hz, H-2'), 7.16 (dq, J=7 Hz, J=1.3 Hz, H-4); ^{13}C NMR in Table I; EIMS (rel. int.): 208 [M]+ (58), 177 $[M-CH_3O]^+$ (33), 176 $[M-CH_3OH]^+$ (30), 149 $[M-C_2H_3O_2]^+$ (100), 109 $[C_6H_5O_2]^+$ (22), 53 $[C_4H_5]^+$ (33).

Gibepyrone E (**6**). Hexane-Et_2O 7:3; 3 μg/ml; oil; $[\alpha]_D$ -3.5; IR (neat): ν 2929, 1720 (C=O), 1640 (C=C), 1270, 840 cm^{-1}; ^1H NMR (300 MHz): δ 1.36 (d, J=5.5 Hz, H-3'), 1.54 (s, $C\underline{H}_3$-C-1'), 2.06 (d, J=1.3 Hz, $C\underline{H}_3$-C-3), 3.10 (q, J=5.5 Hz, H-2'), 6.12 (d, J=6.8 Hz, H-5), 7.08 (dq, J=6.8 Hz, J=1.3 Hz, H-4); ^{13}C NMR

in Table I; EIMS (rel. int.): 180 $[M]^+$ (21), 165 $[M-CH_3]^+$ (1), 152 $[M-CO]^+$ (1), 151 $[M-CHO]^+$ (1), 136 $[M-CO_2]^+$ (42), 121 (100), 109 $[C_6H_5O_2]^+$ (20), 108 (22), 107 (24), 79 (26), 65 (10), 53 $[C_4H_5]^+$ (35). Preparation of **6** and **7**: a solution of m-chloroperbenzoic acid in CH_2Cl_2 (170 mg/4ml) was added to a mixture containing **1** in CH_2Cl_2 (113 mg/3 ml) and 0.5M $NaHCO_3$ (3 ml) and stirred for 30 min. The organic layer was washed, dried, and concentrated to give the crude product which was chromatographed in a silica gel column. **6** (20%) and **7** (24%) were isolated.

Gibepyrone F (**7**). Hexane-Et_2O 85:15; 2 μg/ml; oil; IR (neat): ν 2926, 1701 (C=O), 1689 (C=O), 1597, 1427, 1376, 1342, 1274, 1123, 1085, 861, 755 cm^{-1}; ^1H NMR (300 MHz): δ 2.18 (d, J=1.3 Hz, C<u>H</u>$_3$-C-3), 2.50 (s, C<u>H</u>$_3$-C(O)-), 6.95 (d, J=6.7 Hz, H-5), 7.24 (dq, J=6.7 Hz, J=1.3 Hz, H-4); ^{13}C NMR in Table I; EIMS (rel. int.): 152 $[M]^+$ (33), 137 $[M-CH_3]^+$ (1), 109 $[C_6H_5O_2]^+$ (99), 81 (12), 53 $[C_4H_5]^+$ (100).

Synthesis of gibepyrone C (**3**)

PDC[19] (52 mg) was added to a solution of **2** (25 mg) in CH_2Cl_2 (2 ml). The mixture was stirred for 15 min., then filtered on silica gel and concentrated to give 23 mg of crude product, made up of compounds **3** (yield 65%); oil; ^1H NMR (80 MHz):δ 2.12 (d, J=1 Hz, C<u>H</u>$_3$-C-3), 2.33 (d, J=1 Hz, C<u>H</u>$_3$-C-1'), 6.49 (d, J=7 Hz, H-5), 6.74 (dq, J=7 Hz, J=1 Hz, H-2'), 7.14 (dq, J=7 Hz, J=1 Hz, H-4), 9.16 (d, J=7 Hz,H-3'); and **7** (yield 30%).

Reaction of **1** *with* SeO_2*: obtention of* **11** *and* **12**.

A solution of SeO_2 (159 mg) in ethanol (8 ml) was added dropwise to an ethanolic solution of **1** (110 mg/1 ml). The mixture was stirred for 5 h at room temperature. After removing the ethanol, 30 ml of water were added to the residue, which was then extracted with EtAcO. The organic layer was washed with a saturated solution of sodium bicarbonate, the solvent removed and the crude product chromatographed on silica gel column to give (1'*E*)-6-(1-hydroxymethylpropenyl)-3-methyl-α-pyrone (**11**); eluted with hexane-Et_2O 25:75; oil; yield 10%; IR (neat): ν 3430 (OH); 1720 (C=O) cm^{-1}; ^1H NMR (300 MHz): δ 1.92 (d, J=7.4 Hz, H-3'), 2.08 (d, J=1 Hz, C<u>H</u>$_3$-C-3), 4.44 (s, C<u>H</u>$_2$OH), 6.30 (d, J=7 Hz, H-5), 6.68 (br q, J=7.4 Hz, H-2'), 7.12 (dq, J=7 Hz, J=1 Hz, H-4); ^{13}C NMR in Table I; and (1'*Z*)-6-(1-hydroxymethylpropenyl)-3-methyl-α-pyrone (**12**), hexane-Et_2O 25:75; oil; yield 10%; IR (neat): ν 3430 (OH); 1720 (C=O) cm^{-1}; ^1H NMR (300 MHz): δ 1.95 (d, J=7.3 Hz, H-3'), 2.10 (d, J=1.2 Hz, C<u>H</u>$_3$-C-3), 4.33 (s, C<u>H</u>$_2$OH), 6.06 (br q, J=7.3 Hz, H-2'), 6.19 (d, J=6.8 Hz, H-5), 7.15 (dq, J=6.8 Hz, J=1.2 Hz, H-4); ^{13}C NMR in Table I.

Preparation of **13**, **14**, **15** *and* **16**.

A solution of **8** (250 mg) in acetone (1ml) was added under inert atmosphere to a mixture of N-methylmorpholineN-oxide (195 mg), water (1.4 ml), acetone (1ml) and aqueous 2% OsO_4 (0.6 ml). This solution was stirred for 12 h and then an aqueous solution of $NaHSO_3$ (15 mg/3 ml) was added. The reaction mixture was neutralized with 1N H_2SO_4 and acetone was then removed. The residue was acidified (pH=2) with 2N HCl and extracted with EtAcO. Evaporation of the solvent and chromatography of the residue on silica gel column, eluting with mixtures of Et_2O-EtAcO of increasing polarity, gave two racemic mixtures: (1'*SR*, 2'*RS*)-6-(2',3'-dihydroxy-1'-methylpropyl)-3-methyl-α-pyrone (**13**); Et_2O-EtAcO 1:1; oil; yield 31%, IR (neat): ν 3397 (OH), 2926, 1696 (C=O), 1640 (C=C), 1577, 1452, 1382, 1114, 1054, 994, 904, 821, 764 cm^{-1}; ^1H NMR (300

MHz): δ 1.19 (d, J=7.1 Hz, C$\underline{H}_3$-C-1'), 2.02 (d, J=1.1 Hz, C$\underline{H}_3$-C-3), 2.73 (dq, J=7.7 Hz, J=7.1 Hz, H-1'), 3.54 (dd, J=11.4 Hz, J=6.5 Hz, H-3'$_a$), 3.73 (dd, J=11.4 Hz, J=3 Hz, H-3'$_b$), 3.86. (ddd, J=7.7 Hz, J=6.5 Hz, J=3 Hz, H-2'), 6.01 (d, J=6.7 Hz, H-5), 7.08 (dq, J=6.7 Hz, J=1.1 Hz, H-4); ^{13}C NMR in Table I; EIMS (rel. int.): 198 [M]$^+$ (4), 167 [M-CH_3O]$^+$ (5), 151 (4), 138 [M-$C_2H_4O_2$]$^+$ (100), 109 [$C_6H_5O_2$]$^+$ (30), 95 (31), 53 [C_4H_5]$^+$ (47); and (1'*SR*, 2'*SR*)-6-(2',3'-dihydroxy-1'-methylpropyl)-3-methyl-α-pyrone (**14**); Et$_2$O-EtAcO 1:1; oil; (yield 31%), ^{1}H NMR (300 MHz): δ 1.26 (d, J=7 Hz, C$\underline{H}_3$-C-1'), 2.03 (d, J=1.2 Hz, C$\underline{H}_3$-C-3), 2.69 (dq, J=7 Hz, J=7 Hz, H-1'), 3.47 (dd, J=11.4 Hz, J=7 Hz, H-3'$_a$), 3.58 (dd, J=11.4 Hz, J=3.4 Hz, H-3'$_b$), 3.92 (ddd, J=7 Hz, J=7 Hz, J=3.4 Hz, H-2'), 6.00 (d, J=6.7 Hz, H-5), 7.08 (dq, J=6.7 Hz, J=1.2 Hz, H-4); ^{13}C NMR in Table I. Trityl chloride (272 mg), 4-N,N-dimethylaminopyridine (5.41 mg) and Et_3N (5 ml) in CH_2Cl_2 (5ml) were added to an equimolecular mixture of **13** and **14** (176 mg),. The mixture was stirred under inert atmosphere for 4 h and was then poured into a ice-water bath and extracted with CH_2Cl_2. The organic layer was washed with saturated ammonium chloride solution and evaporated to obtain the reaction crude (340 mg), which contained the derivatives **15** ^{1}H NMR (300 MHz): δ 1.06 (d, J=7.1 Hz, C$\underline{H}_3$-C-1'), 2.03 (d, J=1.2 Hz, C$\underline{H}_3$-C-3), 2.78 (dq, J=7.1 Hz, J=7.1 Hz, H-1'), 3.11 (dd, J=9.7 Hz, J=5.9 Hz, H-3'$_a$), 3.35 (dd, J=9.7 Hz, J=3.7 Hz, H-3'$_b$), 3.97 (ddd, J=7.1 Hz, J=5.9 Hz, J=3.7 Hz, H-2'), 5.93 (d, J=6.6 Hz, H-5), 7.00 (dq, J=6.6 Hz, J=1.2 Hz, H-4), 7.28 (m, 3 Ph); ^{13}C NMR in Table I; and **16** ^{1}H NMR (300 MHz): δ 1.20 (d, J=7 Hz, C$\underline{H}_3$-C-1'), 2.02 (d, J=1.2 Hz, C$\underline{H}_3$-C-3), 2.78 (dq, J=7 Hz, J=6 Hz, H-1'), 3.09 (dd, J=9.6 Hz, J=6 Hz, H-3'$_a$), 3.19 (dd, J=9.6 Hz, J=4.3 Hz, H-3'$_b$), 4.05 (ddd, J=6 Hz, J=6 Hz, J=4.3 Hz, H-2'), 5.83 (d, J=6.7 Hz, H-5), 5.93 (dq, J=6.7 Hz, J=1.2 Hz, H-4), 7.28 (m, 3 Ph); ^{13}C NMR in Table I.

Antimicrobial screening

The antimicrobial activity of gibepyrones A, B, E, and F were tested against Gram positive bacteria (*Enterococcus faecalis* S 48, *Bacillus subtilis* CECT 397, *Staphylococcus aureus* ATCC 8), Gram negative (*Salmonella tiphymurium* LT 2, *Escherichia coli* U 9, *Proteus sp.*) and yeasts (*Saccharomyces cerevisiae* S 3, *Candida albicans* CECT 1394). The microorganisms were obtained from the Microbiology Department, Faculty of Sciences, University of Granada. The minimal inhibitory concentration (MIC) was measured in 1 ml of nutrient broth (tryptose broth ADSA-MICRO for bacteria, and USP ADSA-MICRO Sabouraud medium for yeasts) containing the sample at the required concentration. The test tubes were inoculated with 10^4 cells of the microorganism and incubated at 28ºC (24 h for bacteria and 48 h for fungi). The test tubes were then examined, taking as MIC the least concentration showing no turbidity.

ACKNOWLEDGEMENTS

We thank Professor E. Cerdá-Olmedo (Department of Genetic and Biotecnia, University of Sevilla) for his suggestions, Dra. M. Maqueda for her collaboration in antimicrobial analysis, A. Barragan for the perfomance of HPLC chromatograms and the CICYT for financial support (project no. BIO90-0631).

REFERENCES

1. Bearder, J. R.. *The Biochemistry and Physiology of Gibberellins*; Crozier, A.; Praeger: New york. 1983; pp 251-387.

2. Pitel, A.W.; Vining L. C. *Can. J. Biochem.* **1970**, 48, 623-630.
3. Cross B. E., Webster, G. R. B. *J. Chem. Soc. (C).* **1970**, 1839-1842.
4. Cross B. E., Myers, P. L., Webster, G. R. B. *J. Chem. Soc. (C).* **1970**, 930.
5. Kjaer, D.; Kjaer, A.; Pedersen, C.; Bu'lock, J. D.; Smith, J. R. *J. Chem. Soc. (C).* **1971**, 2792-2797.
6. Pitel, A.W.; Arsenault, G. P.;Vining L. C. *J. Antibiotic.* **1971**. XXIV, 483-484.
7. Springer, J. P.; Clardy, J.; Cole, R. J.; Kirksey, J.W.; Hill, R. K.; Carlson, R.M.; Isidor, J.L. *J. Am. Chem. Soc.* **1974**, 96, 2267-2268.
8. Nes, W. D.; Heupel, R. C.; Le, P. H. *J. Chem. Soc. Chem. Commun.* **1985**, 1431-1433.
9. Avalos, J.; Cerdá-Olmedo, E. *Curr. Genet.* **1987**, 11, 505-511.
10. Barrero, A. F.; Sánchez, J.; Oltra, J. E.; Tamayo, N.; Cerdá-Olmedo, E.; Candau, R.; Avalos, J. *Phytochemistry.* **1991**, 30, 2259-2263.
11. Leslie, J. F.; Dol, F. J.; Plattner, R. D.; Shackelford, D. D.; Jonz, J. *Mycopathologia.* **1992**, 117, 37-45.
12. Geissman, T. A.; Verbiscar, A. J.; Phinney, B. O.; Cragg, J. *Phytochemistry.* **1966**, 5, 933-947.
13. Nair, M. S. R.; Carey, S. T. *Tetrahedron Lett.* **1975**, 1655-1658.
14. Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tabellen Zur Strukturanfklarüng Organischer Verbindungen mit Spektroskopischen Metoden*; Springer-Verlag: Berlin-Heidelberg-New York. 1976.
15. Abraham, W. R.; Arfmann, H. A. *Phytochemistry.* **1988**, 27, 3310-3311.
16. Rey, M.; Dunkelblum, E.; Allain, R.; Dreiding, A. S. *Helv. Chim. Acta.* **1970**, 53, 2159-2175.
17. Avent, A. G.; Hanson, J. R.; Truneh, A. *Phytochemistry.* **1992**, 31, 1065-1066.
18. Abraham,W. R.; Knoch, I.; Witte, L. *Phytochemistry.* **1990**, 29, 2877-2888.
19. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399-402.
20. Barrero, A. F.; Oltra, J. E.; Cabrera, E.; Herrador, M. M.; Rojas, F. J.; Reyes, J. F.; Godoy, F. *Nat. Prod Letters.* (In press).