COMMUNICATIONS TO THE EDITOR

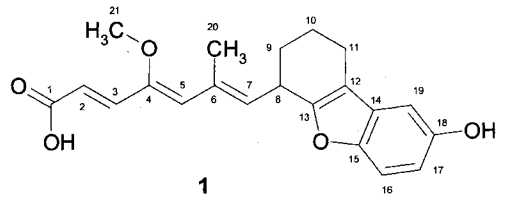
Cuevaenes A and B, New Polyene Carboxylic Acids from *Streptomyces* sp. HKI 0180

Sir:

In the course of a screening for new metabolites from strains of actinomycetes we disclosed *Streptomyces* sp. HKI 0180 as the producer of cuevaenes A (**1**) and B (**2**). The strain was isolated from a soil sample from the cave of Altamira (Spain). In this paper we report the production, isolation, structure elucidation and biological properties of **1** and **2** (Fig. 1). An agar slant culture of the above strain was used to inoculate a 500 ml Erlenmeyer flask containing 100 ml of a seed medium composed of (g/liter): dextrose 15, soya bean meal 15, NaCl 5, $CaCO_3$ 1, KH_2PO_4 0.4, distilled water, pH 6.9. The producing medium contained (g/liter): mannitol 20, soya bean meal 20, distilled water, pH 6.5.

Cultivation occurred at 28°C for 120 hours on rotary shakers (180 r.p.m.) in 1 liter Erlenmeyer flasks containing each 250 ml of medium. 10 liters of the fermentation broth were extracted twice by ethyl acetate (1 : 1). The combined ethyl acetate extracts were dried and evaporated. The residue was chromatographed on Sephadex LH-20 (MeOH) and on silica gel 60 ($CHCl_3$/MeOH, 9 : 1). Cuevaenes were detected by reddish staining with 1% vanillin in conc. H_2SO_4. Final purification was achieved by preparative TLC on silica gel aluminium sheets (Merck, $CHCl_3$/MeOH, 9 : 1). Yield: 15 mg cuevaene A (**1**), 12 mg cuevaene B (**2**). The physico-chemical properties of the new metabolites **1** and **2** (see Fig. 1) are shown in Table 1. The presence of double bonds and carbonyl groups in **1** and **2** was suggested by IR absorbances in the range of 1608~1679 cm^{-1}. The

Fig. 1. Structures (relative stereochemistry) of cuevaenes A (**1**) and B (**2**) from *Streptomyces* sp. HKI 0180.



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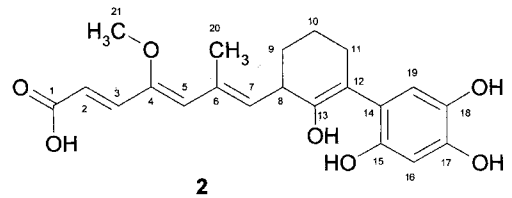

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Table 1. Physico-chemical properties of cuevaenes A (**1**) and B (**2**).

	1	**2**
Appearance	wax	wax
Molecular weight	354	388
Formula	$C_{21}H_{22}O_6$	$C_{21}H_{24}O_7$
Mass spectrum	*m/z* 355.1595 $[M+H]^+$; (calcd. 355.1641 for $C_{21}H_{23}O_5$)	*m/z* 388.1087; M^+; (calcd. 388.0834 for $C_{21}H_{25}O_7$)
IR (λ_{max}, cm^{-1}, KBr)	801, 842, 925, 972, 1036, 1063, 1093, 1136, 1191, 1272, 1370, 1373, 1378, 1450, 1608, 1613, 1679, 2935, 3395	809, 981, 1070, 1091, 1135, 1180, 1265, 1375, 1380, 1495, 1615, 1620, 1679, 2933, 3390
$[\alpha]_D^{25}$ (4 mg/ml MeOH)	+ 88.9 °	+ 95.3 °
R_f (TLC, silica gel sheets, $CHCl_3$, MeOH, 9:1)	0.45	0.35

Table 2. Assignment of ^{1}H and ^{13}C NMR spectra of **1** and **2**.

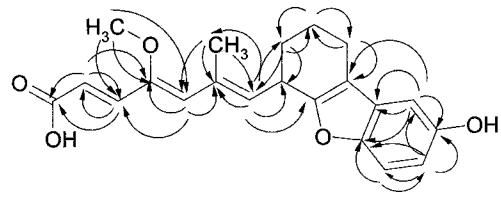
position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	170.5	-	171.0	-
2	118.1	6.0, d, 16.0	118.3	6.01, d, 16.0
3	143.7	7.06, d, 16.0	143.6	7.05, d, 16.0
4	153.4	-	153.6	-
5	130.9	5.95, s	130.7	5.95, s
6	133.0	-	133.2	-
7	140.8	5.69 d, 10.1	140.2	5.75, d, 10.1
8	33.5	3.85, d,d,d, 10.1, 2.2, 4.1	33.5	3.78, d,d,d, 10.1; 2.3; 4.1
9	31.0	1.59 m; 2.01 m	30.9	1.55 m; 2.02 m
10	22.5	1.85 m; 2.08 m	22.4	1.8 m; 2.05 m
11	24.3	2.69 m; 2.71 m	24.2	2.62 m; 2.65 m
12	115.6	-	115.8	-
13	156.1	-	156.8	-
14	130.3	-	129.2	-
15	153.7	-	149.7	-
16	117.7	7.16, d, 9.1	112.6	7.35, s
17	112.3	6.62, d,d, 9.1; 2.5	149.8	-
18	150.3	-	149.6	-
19	105.3	6.7, d, 2.5	106.1	6.85, s
20	15.3	2.24, s	15.4	2.2, s
21	60.7	3.62, s	60.6	3.6, s

in $CDCl_3$, TMS as internal standard, δ in ppm, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad, coupling constants in Hz.

molecular weights and chemical formulas of Table 1 were readily suggested by HRFAB, HREI and ESI mass spectra, showing the M^+, $[M+H]^+$ and $[M+Na]^+$ ions, respectively. The pseudomolecular ions observed during 'soft' ionization (ESI-MS) suggested that **1** contains a tetrahydrodibenzofurane moiety.

The structures of **1** and **2** as depicted in Fig. 1 were settled on the basis of one and two-dimensional ^{1}H and ^{13}C NMR measurements (COSY, TOCSY, DEPT, HSQC, HMBC, NOESY). The ^{1}H NMR spectra of **1** and **2** displayed two (*E*)-configurated olefinic protons (H-2 and H-3, doublets, $^{3}J_{H,H}=16$ Hz), one singlet and one additional doublet olefinic proton (H-5, H-7), and aromatic protons displaying *ortho*- and *meta*-couplings with 9 Hz and 2.5 Hz, respectively, in compound **1**. However, the weak couplings of the two aromatic protons (<0.3 Hz) in **2** suggested their *para* position. Due to the presence of a carboxyl proton the phenolic protons were invisible in the NMR spectra even in $CHCl_3$ or DMSO solution (Table 2). The ^{13}C NMR spectra of **1** and **2** (Table 2) showed for each compound one carbonyl signal (C-1: 170.5 and 171.0 ppm), one methoxyl signal (C-21: 60.7 and 60.8 ppm), one methyl group (C-20, 15.3 and 15.4 ppm) and a series of olefinic and aromatic carbons as shown in Fig. 1.

Fig. 2. Instructive C,H long-range couplings in the HMBC spectrum of **1**.



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For structural assignment the $^{1}H,^{1}H$ COSY and C,H long-range correlated heteronuclear NMR spectra (HMBC) were particularly helpful. Thus the proton and carbon positions for **1** were unambiguously settled as shown in Fig.

2. The all-(*E*)-configuration of the olefinic side chain double bonds in **1** and **2** was confirmed by $^{3}J_{H\text{-}2,H\text{-}3}=15$ Hz, and the strong NOE's observed between H-3/H-5 and H-5/H-7, respectively (Fig. 2).

1 and **2** displayed moderate antibacterial activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* SG511 in concentration $>50\,\mu$g/ml. **1** and **2** thus appear as new members of the non-lactone family of polyene metabolites[1~3].

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