JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Diverse Metabolic Profiles of a *Streptomyces* Strain Isolated from a Hyper-arid Environment

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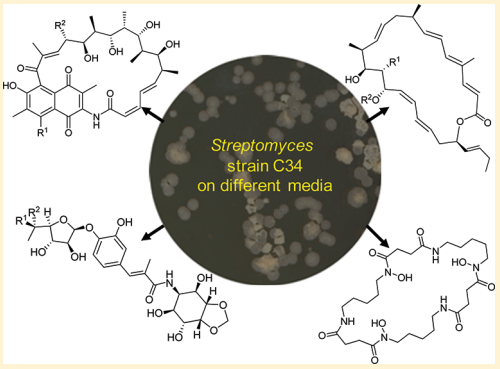
Supporting Information

ABSTRACT: The metabolic profile of *Streptomyces* sp. strain C34, isolated from the Chilean hyper-arid Atacama Desert soil, is dependent on the culture media used for its growth. The application of an OSMAC approach on this strain using a range of cultivation media resulted in the isolation and identification of three new compounds from the rare class of 22-membered macrolactone polyketides, named chaxalactins A−C (**1**−**3**). In addition, the known compounds deferroxamine E (**4**), hygromycin A (**5**), and 5″-dihydrohygromycin A (**6**) were detected. The isolated compounds were characterized by NMR spectroscopy and accurate mass spectrometric analysis. Compounds **1**−**3** displayed strong activity against Gram-positive but weak activity Gram-negative strains tested.



Microbial secondary metabolites are widely considered to be one of the best reservoirs for drug discovery from natural sources.[1] Searching for natural products from terrestrial microorganisms was initiated at least six decades ago and revealed that microbes of soil origin are a prolific supply for structurally unique and bioactive secondary metabolites.[2] *Streptomyces* is the largest genus of the *Actinobacteria*, with over 500 species found predominantly in soil and marine ecosystems.[3] To date *Streptomyces* species are responsible for about two-thirds of the clinically used antibiotics, and so chemical screening of novel *Streptomyces* isolates recovered from unusual environments for new natural products is of great interest.[4,5] Selection of bacterial strains from the underexplored extremobiosphere[5] provides one option, while genome mining based on PCR targeting of specific microbial genes provides an effective route for discovering new natural products.[6] OSMAC (one strain−many compounds) is the approach that we have deployed in this study. This strategy was developed in the early 2000s by Zeeck and co-workers to maximize the productivity of a single microbe probably through turning on silent or cryptic biosynthetic genes by varying accessible cultivation parameters such as media composition, pH, temperature, oxygen supply, quality and quantity of light, bioreactor platform, or the addition of precursors or enzyme inhibitors.[7] Using OSMAC, Zeeck's group isolated more than 100 metabolites representing 25 structural classes from only six microorganisms.[7] The OSMAC approach has been successfully applied to produce new secondary metabolites from single microbial strains during the last 10 years.[8−10]

In the past, researchers hypothesized that extremophiles would not grow or produce natural products unless complex cultivation methods were used.[1] Recently, many examples show that microbes from extreme environments can grow and produce new secondary metabolites under standard laboratory conditions.[1,11] To date, most attention has been applied to marine ecosystems, especially deep sea environments, as a rich source of novel microbes and hence natural products. One of the less commonly investigated habitats is the hyper-arid or absolute deserts, which constitute large landmasses characterized by very low or zero vegetation and extremely low and unpredictable rainfall.[5]

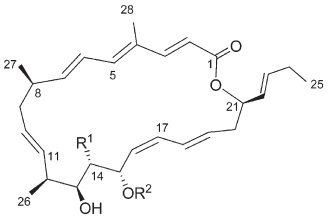
As part of our program to investigate extremophiles as an underexplored source of new natural products, we have focused our attention on *Streptomyces* sp. strain C34 isolated from the Atacama Desert, Chile. This strain was previously shown by our group to contain a 3-amino-5-hydroxybenzoic acid (AHBA) synthase gene,[6] and its fermentation led to the production of four new ansamycin-type polyketides.[6] The application of the

Received: June 7, 2011
Published: August 31, 2011

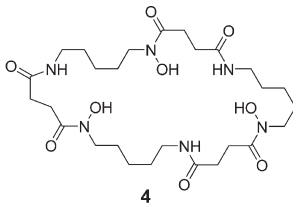
OSMAC approach to this strain using eight different media resulted in the production of three new 22-membered macrolactone-type polyketides, chaxalactins A−C (**1**−**3**), in addition to the known cyclic peptide deferroxamine E (**4**) and the known glycosides hygromycin A (**5**) and 5″-dihydrohygromycin A (**6**). The macrolactones reported in the present study were screened for antibacterial activity against a panel of Gram-positive and Gram-negative bacterial strains.

RESULTS AND DISCUSSION

In our previous study, the taxonomically unique *Streptomyces* sp. strain C34 was induced to produce four new ansamycin-type compounds when cultivated on two different media.[6] In the present study, we have further investigated the LCMS metabolite profiles of this strain when cultured on another eight different media. Changing culture media composition not only affected the produced compounds quantitatively but also had a significant effect on activation of the biosynthetic pathways leading to the production of new metabolites from different chemical classes. Analysis of the chromatograms for these 10 different cultivation media (Figure 1) clearly depicted a dramatic change in the chemical profiles. Cultivation on yeast extract glucose (GYE),[12] soya flour mannitol (SFM),[13] and potato dextrose (PD)[14] media resulted in the production of a sole metabolite, chaxamycin A (**7**), which was also found as a predominant metabolite in all of the other media used. As a result, the previously reported chaxamycin A (**7**)[6] is considered a useful chemical marker of this strain. Chaxamycin B (**8**) was produced in ISP2 and malt extract peptone (MEP)[15] and modified Fries media 1[16] and 2.[16] Production of chaxamycins C and D (**9**, **10**) was exclusive to the modified ISP2 medium. The use of a defined medium[17] led to the discovery of three new metabolites, chaxalactins A−C (**1**−**3**), belonging to the rare microbial class of 22-membered macrolactones, in addition to the cyclic peptide deferroxamine E (**4**). The latter, a known siderophore, seemed to predominate when using media containing a high content of inorganic salts; that is, it was produced as a major metabolite in addition to the known glycosides hygromycin A (**5**) and 5″-dihydrohygromycin A (**6**) when using ISP3 medium[18] (Figure 1).



	R^1	R^2
1	H	H
2	OH	H
3	OH	CH_3



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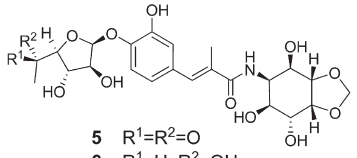
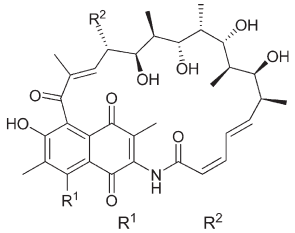
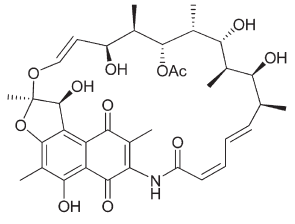


5 R^1=R^2=O
6 R^1=H, R^2=OH



	R^1	R^2
7	OH	CH_3
8	H	CH_3
9	OH	CH_2OH



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HRESIMS analysis of **1** established the molecular formula as $C_{28}H_{40}O_4$, thus implying nine degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 1) revealed the presence of one ester carbonyl group at δ_C 166.6, 14 olefinic carbons, and 13 olefinic protons, consistent with the presence of six disubstituted double bonds and one trisubstituted olefinic group, three oxymethine protons, three methylene groups, and four methyl groups. This analysis brought the total number of unsaturations to eight, thus requiring the presence of one additional ring in the structure.

In the COSY spectrum, two spin systems were discernible, comprising H-2 and H-3 as well as H-5 through H_3-25, including the methyl groups H_3-26 and H_3-27 (Figure 2). The chain C-5−C-25 was further confirmed by HMBC correlations (Table 1). The attachment of methyl group H_3-28 (δ_H 1.81) to the trisubstituted olefinic group was evident from its correlations to C-3, C-4, and C-5 in the HMBC spectrum. Carbonyl group C-1 (δ_C 166.6) was connected to C-2 on the basis of its HMBC correlations to both H-2 (δ_H 5.73) and H-3 (δ_H 7.14), while the HMBC correlation of H-21 (δ_H 5.32) to C-1 allowed for closing of the lactone ring in the proposed structure.

The configuration of the double bonds at C-2−C-3, C-18−C-19, and C-22−C-23 was determined to be *E*, while C-16−C-17 was established as *Z* on the basis of the values of the coupling constants. The double bond at C-4−C-5 was confirmed to be *E* on the basis of the NOESY correlations of H-3 to H-5 and H_3-28 to both H-2 and H-6. The configuration of the remaining double bonds was determined to be *E*, and the relative configuration at the chiral carbons was established as C-8 *R**, C-12 *S**, C-13 *R**, C-15 *R**, and C-21 *R** on the basis of X-ray crystallographic analysis of **1**, while it was not possible to determine the absolute configuration. With respect to the atoms making up the ring, the conformation of the C-8−C-9 bond was found to be *gauche* [C-7−C-8−C-9−C-10 = −64.2(3)°], as is C-12−C-13 [C-11−C-12−C-13−C-14 = −63.8(3)°], whereas C-13−C-14 [C-12−C-13−C-14−C-15 = −171.5(2)°] (Figure 3). The torsion angle of C-16−C-17−C-18−C-19 is 170.1(3)°, indicating the conformation of C-17−C-18 to be *s-trans*. With respect to the side-chain junction (C-21), the ring atoms were found to be *gauche* [C19−C20−C21−O1 = 56.0(3)° and the side chain was *anti* to C-19 [C-19−C-20−C-21−C-22 = 177.4(2)°].

In the crystal, the molecules are linked by O−H···O hydrogen bonds to generate (001) sheets (Figure 4). Atom O4 serves as both a donor and acceptor in this network. In graph-set notation, C(14) chains in [100] and C(6) chains in [010] occur. There were no other intermolecular interactions detected except van der Waals contacts.

A NOESY spectrum was acquired as a reference for the assignments of the relative configuration of the other related compounds. On this basis, it was shown that **1** was a new 22-membered macrolactone-type polyketide for which the name chaxalactin A was ascribed.

The molecular formula of **2** was determined as $C_{28}H_{40}O_5$, thus having one more oxygen atom than **1**. Comparison of the ^1H and ^{13}C NMR spectra of **2** and **1** (Table 1) revealed that both were closely related and that methylene protons H_2-14 in **1** (δ_C 41.4, δ_H 1.59, δ_H 1.47) were replaced by an oxymethine proton [δ_C 73.2, δ_H 3.31] in **2**. This was corroborated with the COSY correlations of H-14 to both H-13 and H-15 and the HMBC correlation of H-14 to C-12. In the ^1H NMR spectrum of **2**, the vicinal coupling constants between H-13−H-14 was 9.2 Hz, suggesting that H-14 and H-13 displayed an *anti* relationship, while the vicinal coupling constant between H-14−H-15

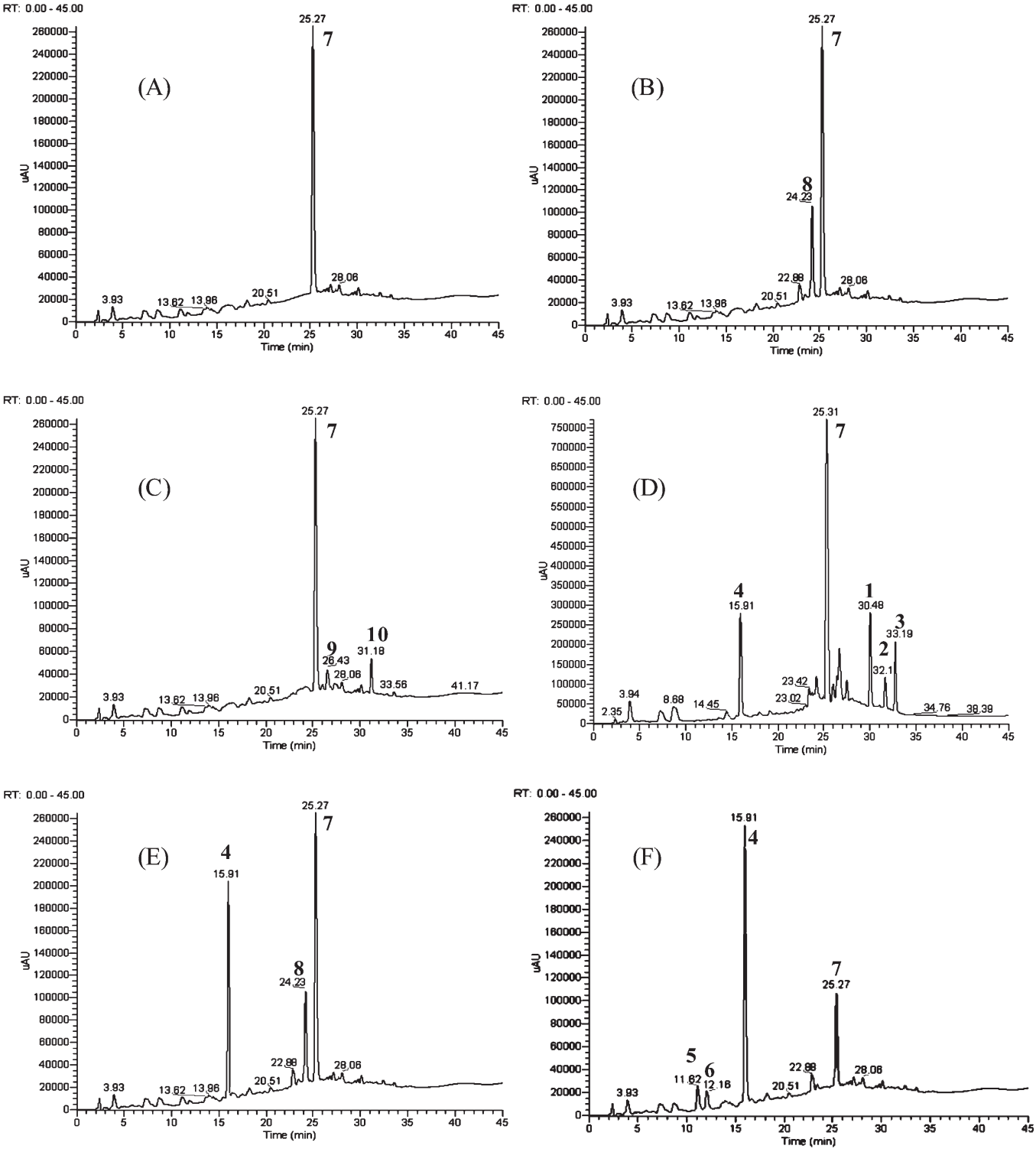

Figure 1. LCMS profiles of the metabolite pattern of *Streptomyces* sp. strain C34 on different media: (A) GYE, SFM, and PD (GYE shown); (B) ISP2 (shown) and MEP; (C) modified ISP2; (D) defined medium; (E) modified Fries media 1 (shown) and 2; (F) ISP3.

was 1.4 Hz, suggesting them to adopt a *gauche* relationship, consistent with a C-13 *S**, C-14 *R**, and C-15 *S** conformation. The close similarity of the ^{13}C NMR data and an analogous set of NOESY correlations for the remaining part of the macrocycle indicated that **2** otherwise had the same relative configuration as **1**. From this analysis, **2** was identified as a new natural product for which we suggested the name chaxalactin B.

HRESIMS analysis of **3** revealed a molecular formula of $C_{29}H_{42}O_5$, indicating **3** to be a homologue of **2**. The NMR spectra of **3** and **2** (Table 1) indicated the presence of an additional methoxyl group in **3** (δ_C 56.2, δ_H 3.27) at position 15 on the basis of the HMBC correlation to C-15 (δ_C75.4). Compounds **2** and **3** displayed similar ^{13}C NMR data and HMBC correlations (Table 1), an analogous set of NOESY

dx.doi.org/10.1021/np200470u |*J. Nat. Prod.* 2011, 74, 1965–1971

Table 1. NMR Spectroscopic Data for Chaxalactins A−C (1−3) ($CDCl_3$, 400 MHz at 298 K)

	chaxalactin A (**1**)			chaxalactin B (**2**)			chaxalactin C (**3**)		
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[a]
1	166.6, C			166.8, C			166.6, C		
2	117.5,CH	5.73, d (15.7)	1,4	117.5, CH	5.73, d (15.6)	1,4	117.2, CH	5.71, d (15.6)	1,4
3	148.5, CH	7.14, d (15.7)	1,2,4,5,28	148.6, CH	7.11, d (15.6)	1,2,4,5,28	148.8, CH	7.13, d (15.7)	1,2,4,5,28
4	132.3, C			132.2, C			132.1, C		
5	138.2, CH	6.19, d (11.2)	3,7,28	138.2, CH	6.13, d (11.1)[a]	3,7,28	138.5, CH	6.14, d (11.2)	3,7,28
6	125.7, CH	6.28, dd (14.7, 11.2)	4,8	125.1, CH	6.27, dd (14.9, 11.2)[b]	5,8	125.2, CH	6.29, dd (14.8, 11.1)[b]	8
7	145.5, CH	5.68, dd (14.7, 8.1)[b]	5,8,27	145.5, CH	5.76, dd (14.9, 7.9)[b]	5,8,27	145.6, CH	5.71, dd (14.8, 8.1)[b]	5,8,27
8	37.5, CH	2.40, m		36.6, CH	2.41, m		36.8, CH	2.42, m	
9	40.9, CH_2	a 2.32, dt (3.7, 12.9)[b]	7	40.9, CH_2	a 2.30 (dt, 3.9, 13.1)[b]		40.9, CH_2	a 2.32, dt (3.8, 12.9)[b]	10
		b 1.89, m	8,11		b 1.98, m	11		b 1.93, m	
10	131.1, CH	5.27, m	12	130.4, CH	5.41, m	12	130.4, CH	5.36, m	
11	133.1, CH	5.27, m	9	132.1, CH	5.42, m	9	132.3, CH	5.44, m	9,12
12	44.0, CH	2.03, m		38.3, CH	2.63, m		38.2, CH	2.60, m	
13	71.8, CH	3.74, dd (10.3, 4.4)	11,15	74.7, CH	3.57, dd (9.4, 2.5)	11,14	74.2, CH	3.62, dd (9.5, 2.6)	11
14	41.4, CH_2	a 1.59, m		73.2, CH	3.31, dd (9.2, 1.7)	12,13	73.7, CH	3.34, dd (9.5, 2.5)	
		b 1.47, dd (14.1, 6.1)	15						
15	65.8, CH	4.80, t (7.5)		67.1, CH	4.79, dd (9.1, 1.4)[b]	17	75.4, CH	4.47, dd (10.6, 2.4)	13,17
16	130.8, CH	5.42, dd (10.5, 7.6)[b]	15,18	127.2, CH	5.65, t (9.4)[b]	18	124.0, CH	5.42, t (10.6)[a]	18
17	131.6, CH	6.04, t (10.6)	15,18,19	133.8, CH	6.17, t (10.6)[b]	15,19	135.1, CH	6.33, t (11.2)	15,18,19
18	129.2, CH	6.61, dd (14.6, 11.7)	17,20	128.7, CH	6.54, dd (14.5, 11.5)	20	128.2, CH	6.54, dd (14.6, 11.4)	17,20
19	131.9, CH	5.64, m	17	133.2, CH	5.68, m	17	133.3, CH	5.40, m	20
20	39.2, CH_2	2.46, m	18,19,21	39.6, CH_2	2.45, m	19,21,22	39.6, CH_2	2.46, m	18,19,21
21	73.0, CH	5.32, m	1	73.0, CH	5.29, m	1,22	72.9, CH	5.27, m	1
22	127.3, CH	5.49, ddt (6.4, 1.4, 15.4)[b]	24	127.4, CH	5.49, ddt (6.5, 1.4, 15.5)[a]	21,24	127.4, CH	5.49, ddt (6.4, 1.5, 15.6)[a]	21
23	135.4, CH	5.79, dt (15.3, 6.3)[b]	21,24,25	135.6, CH	5.82, dt (6.5,15.8)[b]	21,24,25	135.5, CH	5.80, dt (6.4, 15.5)[b]	21,24
24	25.4, CH_2	2.05, m	22,23,25	25.4, CH_2	2.04, m	22,23,25	25.4, CH_2	2.03, m	22,23,25
25	13.4, CH_3	0.97, t (7.4)	23,24	13.4, CH_3	0.97, t (7.4)	23,24	13.4, CH_3	0.96, t (7.4)	23,24
26	17.3, CH_3	1.02, d (6.8)	11,12,13	17.4, CH_3	1.10, d (6.9)	11,12,13	17.6, CH_3	1.09, d (6.9)	11,12,13
27	20.5, CH_3	1.08, d (6.7)	7,8,9	19.8, CH_3	1.09, d (6.7)	7,8,9	20.0, CH_3	1.08, d (6.7)	7,8,9
28	12.5, CH_3	1.81, s	3,4,5	12.5, CH_3	1.81, s	3,4,5	12.5, CH_3	1.80, s	3,4,5
29							56.2, CH_3	3.27, s	15

[a] HMBC correlations are from the proton(s) stated to the indicated carbon(s). [b] Extracted from 1D-TOCSY spectra.

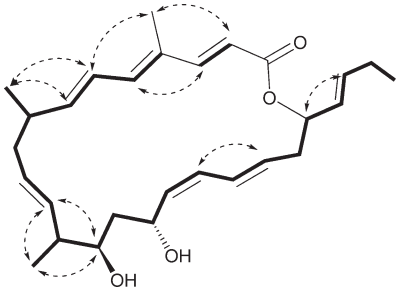

Figure 2. Key COSY (−) and ROESY (↔) correlations of **1**.

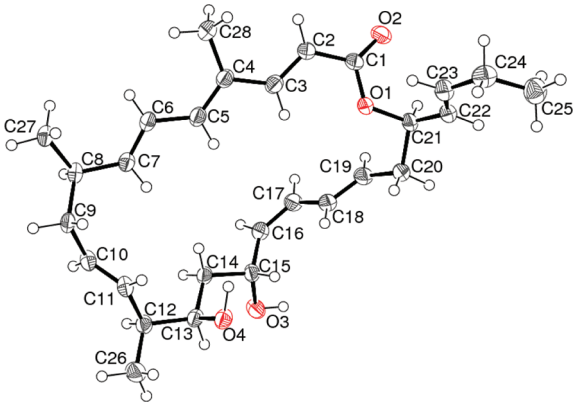

Figure 3. Molecular structure of **1** showing 50% displacement ellipsoids.

correlations, and virtually identical coupling constants between H-13 through H-15, all of which strongly suggested that they shared the same relative configuration. On the basis of this evidence, **3** was confirmed as a new 22-membered macrolactone-type polyketide for which the name chaxalactin C was ascribed.

In Nature, microbial 22-membered macrolactones are rare. They are represented by the antibiotic pulvomycin from *Streptomyces netropsis*,[19] the macrolides A82548a and yokonolide A from *Streptomyces diastatochromogenes*,[20,21] the immunosuppressants ushikulides A and B from *Streptomyces* sp.,[22] the antibacterial

dx.doi.org/10.1021/np200470u |*J. Nat. Prod.* 2011, 74, 1965–1971

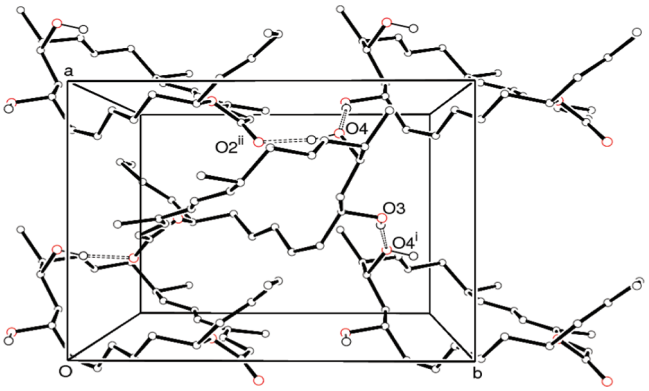

Figure 4. Hydrogen bond interactions in the crystal of **1** leading to (001) sheets. Symmetry codes: (i) $x-1/2, -y+3/2, -z+1$; (ii) $x+1/2, -y+1/2, -z+1$.

difficidin compounds from *Bacillus subtilis*,[23] macrolactin H from *Bacillus* sp.,[24] and the cytotoxic wortmannilactones A–D from the soil-derived fungus *Talaromyces wortmannii*.[25] Chaxalactins A–C (**1**–**3**) are structurally close to the known 24-membered polyketide macrolactin family. To date, macrolactins A–V have been isolated and chemically characterized from marine and soil bacterial isolates[26] and shown to have antibacterial, antilarval, antiviral, and antitumor activities.[26]

This rare group of compounds, produced by the taxonomically distinct *Streptomyces* strain C34,[27] differed from almost all of the structurally related macrolactins in that they were 22-membered rather than 24-membered macrocyclic lactones, with branching methyl groups at C-4, C-8, and C-12, and an *E*-but-1-ene side chain at C-21 instead of a methyl or ethyl group at the corresponding branching position in the 24-membered macrolactins. They also differed from almost all 24-membered lactones in having an *E* configuration at C-2–C-3 instead of *Z* and *Z* instead of *E* at C-16–C-17 and by the sense of optical rotation, i.e., dextrorotatory instead of levorotatory.

Type I multimodular polyketide synthases (type I PKSs) should be involved in the biosynthesis of the macrolide **1**, and the assembly line is likely to utilize eight acetate and four propionate units without any postbiosynthetic modifications (Figure 1, SI). It is clear from the proposed biogeneses that chaxamycins and chaxalactins have different biosynthetic origins.

When tested for antibacterial activity (Table 2), compounds **1**–**3** displayed strong activity against Gram-positive bacteria with MIC values of <1 μg mL^{-1} against *S. aureus* and 3–6 μg mL^{-1} against *L. monocytogenes* and *B. subtilis*, but showed weak activity against Gram-negative strains tested. It was noticed that compound **2** showed slightly higher activity against *S. aureus* than compounds **1** and **3** despite the minor structural differences.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer 343 polarimeter. UV and IR spectra were measured on a Perkin-Elmer Lambda 25 UV/vis spectrometer and a Thermo Nicolet IR 100 FT/IR spectrometer, respectively. ^{1}H, ^{13}C, and 2D NMR experiments were acquired on a Varian Unity INOVA 400 MHz spectrometer. High-resolution mass spectral data were obtained from a Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler, and Accela pump).

Table 2. Antibacterial Activity of Chaxalactins A–C (1–3)

	average MIC (μg mL^{-1})		
strain	1	2	3
S. aureus	0.4	0.2	0.8
L. monocytogenes	6.3	5.0	6.3
B. subtilis	3.1	5.0	6.3
E. coli	>20	>20	>20
V. parahemolyticus	12.5	20	12.5

The following conditions were used: capillary voltage 45 V, capillary temperature 320 °C, auxiliary gas flow rate 10–20 arbitrary units, sheath gas flow rate 40–50 arbitrary units, spray voltage 4.5 kV, mass range 100–2000 amu (maximum resolution 30000). HPLC separations were made using a Phenomenex reversed-phase (C_{18}, 250 × 10 mm, L × i.d.) column connected to an Agilent 1200 series binary pump and monitored using an Agilent photodiode array detector. Detection was carried out at 220, 230, 254, 280, and 350 nm. Diaion HP-20 was obtained from Resindion S.R.L., a subsidiary of Mitsubishi Chemical Co., Binasco, Italy.

Microorganism and Fermentation Conditions. *Streptomyces sp.* strain C34 (EU551711) was isolated from a soil sample from Laguna de Chaxa (Salar de Atacama, Chile).[27] The first-stage seed culture of this strain was prepared by inoculating a single colony of the organism into 50 mL of a defined medium[17] of the following composition (g L^{-1}): KH_2PO_4 (2.0), NH_4Cl (1.5), $MgSO_4 \cdot 7H_2O$ (0.5), NaCl (0.5), glycerol (10), myo-inositol (0.4), monosodium L-glutamate monohydrate (5.0), NaF (0.084), $FeSO_4 \cdot 7H_2O$ (0.025), $ZnSO_4 \cdot 7H_2O$ (0.01), $CoCl_2 \cdot 6H_2O$ (0.01), $CaCO_3$ (0.25), *p*-aminobenzoic acid (0.001), pH 7.0. After 5 days incubation in a rotary shaker incubator at 28 °C at 150 rpm, this culture was used to inoculate the production fermentation, using 3 L of the same medium in the presence of Diaion HP20 (50 g L^{-1}) under the same conditions for 7 days. A wide range of media was used to screen the effects of simple, complex, enriched, or defined media as well as high inorganic salt content on the bacterial secondary metabolite production. The same procedure was adapted in the bacterial fermentation on GYE, SFM, PD, MEP, ISP3, and modified Fries media 1 and 2.

Extraction and Isolation. The harvested fermentation broth from strain *Streptomyces* C34 (defined medium, 3 L) was centrifuged at 3000 rpm for 20 min, and the HP20 resin together with the cell mass were washed with distilled water and then extracted with MeOH (4 × 500 mL). The successive MeOH extracts were combined and concentrated under reduced pressure, yielding 250 mL of materials, which were fractionated with *n*-hexane (3 × 250 mL), CH_2Cl_2 (4 × 250 mL), and EtOAc (3 × 250 mL). Quick NMR analysis revealed that the CH_2Cl_2 fraction had the most diverse metabolic profile. This fraction was loaded on a Sephadex LH-20 column equilibrated with MeOH–CH_2Cl_2 (1:1), and two fractions were collected. The second fraction (210 mg) was further purified by reversed-phase HPLC using a gradient of MeOH in H_2O as eluent (60–100% over 60 min, 100% for 10 min) at a flow rate of 1.25 mL min^{-1}. This procedure afforded the pure compounds **1** (19 mg), **2** (7 mg), **3** (11 mg), and **4** (95 mg) from the defined medium and **5** (1.4 mg) and **6** (1 mg) from ISP3 medium.

Chaxalactin A (***1***): colorless needles (MeOH); mp 89.7 °C; $[\alpha]^{20}_D$ +37 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 229 (3.74), 258 (2.38) nm; IR (film) ν_{max} 3413, 2915, 1708, 1635, 1587 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 463.2815 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_4Na$, 463.2819).

Chaxalactin B (***2***): yellowish-white powder ($CHCl_3$); $[\alpha]^{20}_D$ +43 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 229 (3.64), 259 (2.33) nm; IR (film) ν_{max} 3421, 2919, 1705, 1638, 1591 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 479.2767 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_5Na$, 479.2768).

Chaxalactin C (**3**): yellowish-white powder ($CHCl_3$); $[\alpha]^{20}_D$ +47 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 229 (3.69), 259 (2.29) nm; IR (film) ν_{max} 3427, 2909, 1701, 1629, 1581 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 493.2921 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_5Na$, 493.2924).

X-ray Crystallographic Study of 1. Intensity data for **1** were collected at 120 K using a Bruker−Nonius Kappa CCD diffractometer (graphite-monochromated Mo Kα radiation, λ = 0.71073 Å). The structure was solved by direct methods with SHELXS-97 and the atomic model refined against $|F|^2$ with SHELXL-97. The "observed data" criterion for calculating the $R(F)$ residuals was set as $I > 2\sigma(I)$. The carbon-bound H atoms were geometrically placed (C−H = 0.95−1.00 Å) and refined as riding atoms with $U_{iso}(H) = 1.2-1.5U_{eq}(C)$. The methyl groups were allowed to rotate about their C−C bonds, but not to tip, to best fit the electron density. The O-bond H atoms were located in difference maps and refined as riding atoms in their as-found relative positions with the constraint $U_{iso}(H) = 1.2U_{eq}$(carrier). Anomalous dispersion was negligible, and Friedel pairs were merged before refinement.

Crystal data for 1: $C_{28}H_{40}O_4$, colorless block, 0.38 × 0.28 × 0.18 mm, M_r = 440.60, orthorhombic, $P2_12_12_1$ (No. 19), a = 8.8119(5) Å, b = 11.8088(4) Å, c = 25.8659(15) Å, V = 2691.6(2) $Å^3$, Z = 4, T = 120 K, ρ_{calc} = 1.087 g cm^{-3}, μ = 0.071 mm^{-1}, $F(000)$ = 960, 20 760 reflections measured ($-9 \le h \le 10$, $-14 \le k \le 13$, $-31 \le l \le 31$; $5.86° \le 2\theta \le 52.00°$), R_{Int} = 0.068, 2908 merged reflections, 2367 with $I > 2\sigma(I)$, 296 parameters, $R(F)$ = 0.044, $wR(F^2)$ = 0.094, S (goodness of fit) = 1.046, $w = 1/[\sigma^2(F_o^2) + 0.0312P^2 + 0.8486P]$, where $P = (F_o^2 + 2F_c^2)/3$, min./max. $\Delta\rho$ = −0.16, +0.18 e $Å^{-3}$. Crystallographic data (excluding structure factor tables) for **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 823154. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int.+44(0)(1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk].

Antibacterial Screening. The antibacterial activity of compounds **1**−**3** was evaluated against the Gram-positive strains *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* ATCC19115, and, *Bacillus subtilis* NCTC2116 and the Gram-negative strains *Escherichia coli* ATCC25922 and *Vibrio parahemolyticus* NCTC10441, using the agar diffusion method.[28] Filter paper disks containing ampicillin (10 μg), oxolinic acid (2 μg), or oxytetracycline (30 μg) were used as positive controls. MICs against species were calculated using the method described before with minor modifications.[32] In brief, tested strains were grown in Müller-Hinton (MH) broth[29] to early stationary phase and then diluted to an OD_{600} = 0.005. The assays were performed in a 96-well microtiter plate format in duplicate, with two independent cultures for each strain. All compounds were dissolved in DMSO (Sigma) and added to the cultures in wells so that the final concentration of DMSO was 10%, a concentration that did not affect the growth of any of the tested strains. The effect of different dilutions of the compounds (80−0.08 μg mL^{-1}) on the growth was assessed after 18 h incubation at 37 °C using a Labsystems iEMS reader MF plate reader at OD_{620}. The MIC was determined as the lowest concentration showing no growth compared to the MH broth control.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of **1**−**3**, including ^1H, ^{13}C, COSY, HSQC, HMBC, and NOESY in $CDCl_3$, are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank the Egyptian Government for a Ph.D. scholarship to M.E.R.; A. Crossman, Department of Chemistry, University of Dundee, for determination of the optical activity; A. Raab, Marine Biodiscovery Centre for LCMS; Iain Douglas, Marine Biodiscovery Centre, for his help in the antibacterial screening; and the College of Physical Sciences, University of Aberdeen, for provision of infrastructure and facilities in the Marine Biodiscovery Centre. W.E.H. is the recipient of a SULSA postdoctoral fellowship. A.T.B. thanks the Leverhulme Trust for an Emeritus Fellowship, and A.T.B. and J.A.S. thank the Royal Society for International Joint Project Grant JP100654.

■ REFERENCES

(1) Pettit, R. K. *Mar. Biotechnol.* **2011**, *13*, 1–11.
(2) Gustafson, K.; Roman, M.; Fenical, W. *J. Am. Chem. Soc.* **1989**, *111*, 7519–7524.
(3) Goodfellow, M.; Fiedler, H.-P. *Antonie van Leeuwenhoek* **2010**, *98*, 119–142.
(4) Lam, K. S. *Curr. Opin. Microbiol.* **2006**, *9*, 245–251.
(5) Bull, A. T. In *Extremophiles Handbook*; Horikoshi, K., Ed.; Springer Verlag: Tokyo, 2011; pp 1204−1240.
(6) Rateb, M. E.; Houssen, W. E.; Arnold, M.; Abdelrahman, M. H.; Deng, H.; Harrison, W. T. A.; Okoro, C. K.; Asenjo, J. A.; Andrews, B. A.; Ferguson, G.; Bull, A. T.; Goodfellow, M.; Ebel, R.; Jaspars, M. *J. Nat. Prod.* **2011**, DOI: 10.1021/np200320u.
(7) Bode, H. B.; Bethe, B.; Hofs, R.; Zeeck, A. *Chembiochem* **2002**, *3*, 619–627.
(8) Christian, O. E.; Compton, J.; Christian, K. R.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2005**, *68*, 1592–1597.
(9) Lin, Z. J.; Zhu, T. J.; Wei, H. J.; Zhang, G. J.; Wang, H.; Gu, Q. Q. *Eur. J. Org. Chem.* **2009**, *18*, 3045–3051.
(10) Lin, Z. J.; Zhu, T. J.; Chen, L.; Gu, Q. Q. *Chin. Chem. Lett.* **2010**, *21*, 824–826.
(11) Wilson, Z. E.; Brimble, M. A. *Nat. Prod. Rep.* **2009**, *26*, 44–71.
(12) Gordon, R. E.; Mihm, J. M. *Ann. N. Y. Acad. Sci.* **1962**, *98*, 628–636.
(13) Wang, H. A.; Qin, L.; Lu, P.; Pang, Z. X.; Deng, Z. X.; Zhao, G. P. *Acta Biochim. Biophys. Sin.* **2006**, *38*, 271–280.
(14) Bouras, N.; Strelkov, S. E. *Can. J. Microbiol.* **2010**, *56*, 874–882.
(15) Hames-Kocabas, E. E.; Uzel, A. *Ann. Microbiol.* **2007**, *57*, 71–75.
(16) Echetebu, C. O. *J. Gen. Microbiol.* **1982**, *128*, 2735–2738.
(17) Reid, K. A.; Hamilton, J. T. G.; Bowden, R. D.; Ohagan, D.; Dasaradhi, L.; Amin, M. R.; Harper, D. B. *Microbiology* **1995**, *141*, 1385–1393.
(18) Wu, R. Y. *Bot. Bull. Acad. Sin.* **1993**, *34*, 363–372.
(19) Smith, R. J.; Williams, D. H.; Barna, J. C. J.; McDermott, I. R.; Haegele, K. D.; Piriou, F.; Wagner, J.; Higgins, W. *J. Am. Chem. Soc.* **1985**, *107*, 2849–2857.
(20) Kirst, H. A.; Larsen, S. H.; Paschal, J. W.; Occolowitz, J. L.; Creemer, L. C.; Steiner, J. L. R.; Lobkovsky, E.; Clardy, J. *J. Antibiot.* **1995**, *48*, 990–996.
(21) Hayashi, K.; Ogino, K.; Oono, Y.; Uchimiya, H.; Nozaki, H. *J. Antibiot.* **2001**, *54*, 573–581.
(22) Takahashi, K.; Yoshihara, T.; Kurosawa, K. *J. Antibiot.* **2005**, *58*, 420–424.
(23) Wilson, K. E.; Flor, J. E.; Schwartz, R. E.; Joshua, H.; Smith, J. L.; Pelak, B. A.; Liesch, J. M.; Hensens, O. D. *J. Antibiot.* **1987**, *40*, 1682–1691.
(24) Nagao, T.; Adachi, K.; Sakai, M.; Nishijima, M.; Sano, H. *J. Antibiot.* **2001**, *54*, 333–339.
(25) Dong, Y. S.; Yang, J. S.; Zhang, H.; Lin, J.; Ren, X.; Liu, M.; Lu, X. H.; He, J. G. *J. Nat. Prod.* **2006**, *69*, 128–130.
(26) Gao, C. H.; Tian, X. P.; Qi, S. H.; Luo, X. M.; Wang, P.; Zhang, S. *J. Antibiot.* **2010**, *63*, 191–193, and references therein.

(27) Okoro, C. K.; Brown, R.; Jones, A. L.; Andrews, B. A.; Asenjo, J. A.; Goodfellow, M.; Bull, A. T. *Antonie van Leeuwenhoek* **2009**, *95*, 121–133.
(28) Kronvall, G. *J. Clin. Microbiol.* **1983**, *17*, 975–980.
(29) Müller, H. J.; J., H. *Proc. Soc. Exp. Biol. Med.* **1941**, *48*, 330–333.