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A new furan carboxamide and two potential precursors from a terrestrial streptomycete

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Abstract: Three new bioactive metabolites, 1,6-dihydroxy-2-methyl-heptan-4-one (**1**), 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**2**), and 2-(2-hydroxy-propyl)-4-methylfuran-3-carboxylic acid amide (**3**) were isolated from the terrestrial *Streptomyces* sp. isolate ANK245, along with the new microbial constituent *p*-vinylanisol (**4a**) and the known metabolites *p*-vinyl-phenol (**4b**) and phenethyl alcohol. Analysis of the nonpolar part of the extract by gas chromatography/mass spectrometry (GC-MS) provided further evidence for tetradecanoic acid, 9-octadecenoic acid, hexadecanoic acid, 2-methoxy-4-vinylphenol (**4c**), 4-hydroxy-3-methoxy-benzaldehyde, *o*-hydroxybiphenyl, and 1,5,9-trimethyl-4,8,13-cyclotetradecatrien-1,3-diol (**5**). Structures **1–3** of the new compounds were elucidated by nuclear magnetic resonance (NMR) and NMR spectroscopy, but mass spectrometry (MS) techniques and their absolute configuration were determined by density functional theory (DFT) calculations and Mosher derivatisation. Their antimicrobial and cytotoxic activities were evaluated in comparison with the crude bacterial extract.

Keywords: CD calculations; furan carboxamide; terrestrial *Streptomyces* sp.

1 Introduction

Streptomyces spp. are widespread in nature and play a significant role in the production of bioactive metabolites [1–3]. Many of their secondary products are having a great bio-functional diversity, e.g. as antibiotics, antifungal, antiviral, anticancer, immunosuppressant agents, insecticides, herbicides, etc., and are playing an important role as potentially useful pharmacological and agricultural agents [4–10].

Extracts of the terrestrial *Streptomyces* sp. strain ANK245 exhibited several diversely staining bands (dark red to brown) on thin layer chromatography (TLC) after spraying with anisaldehyde-sulfuric acid and heating. A large-scale fermentation of the strain and separation of its extract using a series of chromatographic techniques led to the isolation of three new products, 1,6-dihydroxy-2-methyl-heptan-4-one (**1**), 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**2**) and 2-(2-hydroxy-propyl)-4-methylfuran-3-carboxylic acid amide (**3**) (see Fig. 1). Additionally, *p*-vinylanisol (**4a**), *p*-vinylphenol (**4b**) and 2-phenylethanol were isolated. In the non-polar part of the mycelium extract, tetradecanoic acid, 9-octadecenoic acid, hexadecanoic acid, 2-methoxy-4-vinylphenol (**4c**), 4-hydroxy-3-methoxy-benzaldehyde, *o*-hydroxybiphenyl, *p*-vinylphenol (**4b**), 1,5,9-trimethyl-4,8,13-cyclotetradecatrien-1,3-diol (**5**), and 1,1′-(*p*-chloro)-bisphenyl sulfone (a widespread pollutant) were identified by gas chromatography/mass spectrometry (GC-MS). The structures of the new compounds **1–4** were solved using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry [chemical ionization (CI), electron ionization (EI), electrospray ionization (ESI) and ESI-HRMS].

2 Results and discussion

Compound **1** was obtained as a moderately polar colorless oil, which was not UV absorbing or fluorescent, but developed on TLC with anisaldehyde-sulfuric acid a dark green color, later changing to dark blue. Pseudomolecular ions of **1** were found by CI-MS and (+)-ESI-MS at 160 $[(M - H_2O) + NH_4]^+$ and 183 Dalton $[M + Na]^+$, respectively, and the molecular formula was established as $C_8H_{16}O_3$ by ESI-HRMS of the $[M + Na]^+$ ion, indicating one double bond equivalent (DBE) (Table 1).

The ^{13}C NMR spectrum displayed eight signals, which were identified by their HSQC data as two methyls, three methylene and two methine groups, and one carbonyl;

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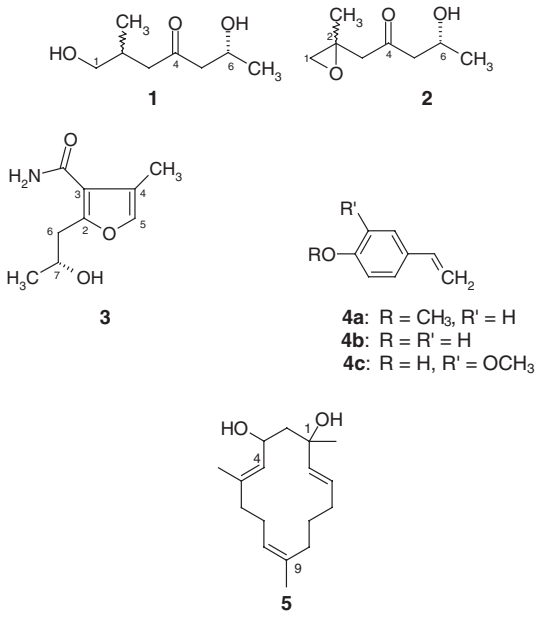

Fig. 1: Structural formulae of compounds **1–5**.

according to the shifts, one methylene and methine group was hydroxylated. The ^{1}H NMR and COSY data of **1** revealed the existence of two separate spin systems, identified as a 2-hydroxypropyl ($-CH_2-CHOH-CH_3$) and a 3-hydroxy-2-methyl-propyl fragment ($HO-CH_2-CH(CH_3)-CH_2-$). According to the number of carbon atoms and the empirical formula, it is evident that these fragments are connected via a remaining carbonyl group. This was confirmed by the ^{13}C NMR data and the HMBC couplings of the carbonyl with the methylene protons CH_2-3 and CH_2-5 and also the methines CH-2 and CH-6 (Fig. 2). Based on these data, **1** was identified as 1,6-dihydroxy-2-methyl-heptan-4-one, a compound, which had not been described before [1, 2, 11].

A closer inspection of the ^{13}C NMR spectrum showed that all carbons were represented by a narrow pair of signals in the ratio of about 0.83 : 1, so that a mixture of diastereomers was expected. Nevertheless, the determination of the configuration was possible by means of Mosher derivatives (see Table S1 in the Supporting Information) [12]. For a mixture of the *rel*-(2*R*/*S*, 6*R*) diastereomers, only one doublet (or a close pair of doublets) should be visible for Me-7, which would give a positive $\Delta\delta_{(S-R)}$ value for the (6*R*) diastereomer, while for the two (2*R*/*S*)-Me signals a negative difference is expected: this is the case for the mixture of diastereomers isolated here (see Figure S7 in the Supporting Information). In contrast, for *rel*-(2*R*,

Table 1: Physico-chemical properties of compounds **1–3**.

	1	2	3
Appearance	Colorless oil	Yellow oil	Colorless solid
R_f	0.40 (CH_2Cl_2-7% MeOH)	0.22 (CH_2Cl_2-7% MeOH)	0.33 (CH_2Cl_2-7% MeOH)
Coloration with anisaldehyde-sulfuric acid	Dark green, turned later to dark blue	Orange, turned later to violet	Pink
Solubility	Soluble in DMSO, MeOH, EtOH EtOAc and CH_2Cl_2. Insoluble in hexane	Soluble in DMSO, MeOH, EtOH EtOAc and CH_2Cl_2. Insoluble in hexane	Soluble in DMSO, MeOH, EtOH, EtOAc, and CH_2Cl_2. Insoluble in hexane
Molecular formula	$C_8H_{16}O_3$	$C_8H_{14}O_3$	$C_9H_{13}NO_3$
(+)-ESI-MS: *m/z* (%)	183 $[M+Na]^+$	–	389 ($[2M+Na]^+$, 100) 206 ($[M+Na]^+$, 44)
EI-MS: *m/z* (%)	143 ($[M-H_2O]^+$, 100), 99 (78), 45 (16), 43 (58), 41 (20)	145 (20), 140 ($[M-H_2O]^+$, 6), 127 (60), 101 (22), 85 (36), 75 (28), 69 (22), 57 (22), 45 (23), 43 (100), 41 (8)	139 ($[M-CONH_2]^+$, 100), 122 (70), 94 (25), 43 (18)
DCI-MS: *m/z* (%)	160 $[M-H_2O+NH_4]^+$	176 ($[M+NH_4]^+$, 100), 159 ($[M+H]^+$, 32)	367 ($[2M+H]^+$, 10), 201 ($[M+NH_4]^+$, 38), 184 ($[M+H]^+$, 100)
(+)-ESI-HRMS			
Found	183.09910 $[M+Na]^+$	181.08368 $[M+Na]^+$	184.09679 $[M+H]^+$ and 206.07876 $[M+Na]^+$
Calcd.	183.09917 for $C_8H_{16}O_3Na$	181.08352 for $C_8H_{14}O_3Na$	184.09682 for $C_9H_{14}NO_3$ 206.07878 for $C_9H_{13}NO_3Na$
UV/Vis: λ_{max} (log ε)	–	–	(MeOH): 241 (3.63); (MeOH–HCl): 242 (3.56); (MeOH–NaOH): 240 (3.62) nm
$[\alpha]_D^{25}$ ($c = 0.1$, MeOH)	– 24°	– 30°	– 42°

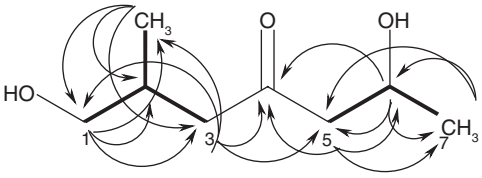

Fig. 2: HMBC (→) and H,H COSY (—) connectivities of 1,6-dihydroxy-2-methylheptan-4-one (**1**).

6*S*/*R*)-**1**, two separated Me-7 signals must be visible, one with a positive, the other one with a negative ($\delta_{(S)}-\delta_{(R)}$) value, which is not found here. This result is confirmed by the carbon shift difference of the signal pairs: while for C-4 to C-8 shift differences between 0.00 and 0.06 ppm are observed, these differences are substantially higher for C-1 (0.07), C-2 (0.25), and C-3 (0.20), so that the epimerisation had certainly occurred at C-2, and **1** has the absolute (2*R*/*S*, 6*R*) configuration. This result was confirmed by the density functional theory (DFT)-calculated optical rotation of – 93° for a 1 : 1 mixture of the (2*R*/*S*, 6*R*) diastereomers, which is therefore unequivocally identified as (2*R*/*S*, 6*R*)-1,6-dihydroxy-2-methyl-heptan-4-one.

A compound **2**, closely related to **1**, was obtained as higher polar yellow oil that gave an orange coloration with anisaldehyde-sulfuric acid, which later turned to violet. No UV activity was visible, an indication for the absence of aromatic and unsaturated system. The molecular weight was determined as 158 Dalton by CI- and ESI-MS, and ESI-HRMS deduced the molecular formula as $C_8H_{14}O_3$, corresponding to two DBEs (Table 1).

The ^{1}H and HMQC spectra of **2** (Table 2) revealed a high similarity with the previous compound, and a 2-hydroxy-*n*-propyl group was identified as in **1** (Fig. 1). In addition, an isobutyl fragment was found; the signal of 2-Me appeared here, however, as a singlet at $\delta = 1.20$ ppm, along with a 2H singlet at $\delta = 3.44$ ppm, corresponding to an oxygenated methylene. The remaining methylene protons (H_2-3) appeared at $\delta = 2.80$ (dd, 16.0, 9.2 Hz) and 2.56 ppm (m), respectively. In accordance, the sole difference between compounds **2** and **1** was attributed to the dehydrogenation during a ring closure between C-1 and C-2 in **1** via oxygen, giving an oxirane ring in **2**. According to this conclusion, the methine carbon C-2 in **1** ($\delta = 31.9$ ppm) had changed into a quaternary oxygenated carbon at $\delta = 72.6$ ppm. Correspondingly, the methyl doublet of 2-CH_3 in **1** ($\delta = 0.87$ ppm) appeared here as singlet ($\delta_H = 1.20$, $\delta_C = 24.3$ ppm), where the downfield shift indicated its neighborhood to the oxygenated C-2. The structure of **2** was finally confirmed by HMBC correlations (Fig. 3), showing for the central 4-CO ($\delta = 212.6$ ppm) the same correlations from both sides. So, the structure of **2** was finally elucidated

Table 2: NMR shift assignments of compounds **1–3** ($CDCl_3$, coupling constants *J* in Hz).

No.	1,6-Dihydroxy-2-methyl-heptan-4-one (1)		Epoxy-heptanone (2)		Furan carboxamide (3)	
	δ_C[a,b]	δ_H[c]	δ_C[a]	δ_H[c]	δ_C[a]	δ_H[c]
1	67.29 67.22	3.44 (dd, 10.6, 5.0), 3.27 (m)	69.3	3.44 (s)		
2	31.87 31.92	2.19 (m)	72.6	–	155.9	–
2-CH_3	16.86	0.87 (d, 6.6)	24.3	1.20 (s)	–	–
3	47.81 47.61	2.50 (m), 2.25 (m)	49.6	2.80 (dd, 16.0, 9.2), 2.56 (m)	118.6	–
3-*C*ONH_2	–	–	–	–	167.2	–
3-CO*N*H_2					–	6.19, 7.24 (s br, NH_2)
4	211.95 211.89	–	212.6	–	119.6	–
4-CH_3	–	–	–	–	9.5	2.13 (d, 1.2)
5	51.33 51.35	2.50 (m)	52.9	2.62 (m)	138.4	7.09 (q, 1.1)
6	63.93 63.99	4.18 (m)	63.9	4.25 (m)	36.6	2.91, 2.97 (AB 14.4, AX 7.3, BX 3.4)
7	22.51 22.56	1.14 (d, 6.2)	22.7	1.20 (d, 6.2)	67.2	4.14 (m)
8	–	–	–	–	23.6	1.26 (d, 6.2)

[a]125 MHz; [b]major signal first (if two signals present); [c]300 MHz.

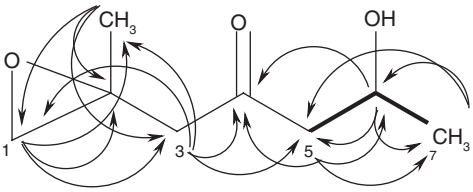

Fig. 3: HMBC (→), and H,H COSY (—) correlations of 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**2**).

as 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one, a new natural product. Also here, the ^{13}C NMR spectrum indicated a mixture of diastereomers. A further determination of the configuration as for **1** was, however, not possible due to rapid decomposition. If the structural similarity of **2** with **1** and **3** is reflecting also a biosynthetic relation, C-6 in **2** may be (*R*)-configured as well, and C-2 is racemic.

Compound **3** was obtained as colorless solid of moderate polarity, showing UV absorbance at 254 nm and a pink color with anisaldehyde-sulfuric acid. The UV spectrum of **3** showed an intense peak at $\lambda_{max} = 242$ nm, indicating a conjugated system, other than in **1** and **2** (Table 1). The molecular weight of **3** was determined as 183 Dalton by ESI-MS, indicating the existence of an odd number of nitrogen atoms in the structure. The corresponding molecular formula was derived as $C_9H_{13}NO_3$ by HRESI-MS, which indicated four DBEs. On EI-MS, the parent molecule lost a fragment with a mass of 44 amu, which was identified by EI-HRMS as a carboxamido group ($CONH_2$) and confirmed by a ^{13}C NMR signal at $\delta = 167.2$ ppm.

The ^{1}H NMR spectrum of **3** displayed a 1H quartet at $\delta = 7.09$ ppm ($J \sim 1.1$ Hz), pointing to a long-range coupling via a double bond with the doublet at $\delta = 2.13$ ppm ($J \sim 1.2$ Hz) of an sp^2-bound methyl group. Two broad 1 H signals due to the amide group were visible at $\delta = 7.24$ and 6.19 ppm. In the aliphatic region, three signals were visible: a methyl doublet at $\delta = 1.26$ ppm ($J \sim 7$ Hz) and a methylene multiplet ($\delta = 2.95$ ppm) both gave COSY correlations (Fig. 4) with the oxymethine multiplet at $\delta = 4.14$ ppm, so that a 2-oxypropyl fragment was established as in **1** and **2**.

In addition to signals of the methyl, carboxamido and propyl fragments as discussed above, four olefinic carbons were identified in the ^{13}C NMR spectrum (Table 2), an oxymethine carbon ($\delta = 138.4$ ppm) and three quaternary atoms. One of the latter was also oxygenated ($\delta = 155.9$ ppm), the other two were found at $\delta = 119.6$ and 118.6 ppm. Based on the remaining oxygen and the left DBE, compound **3** should be a trisubstituted furan, decorated with a methyl, a carboxamido, and a 2-hydroxypropyl group.

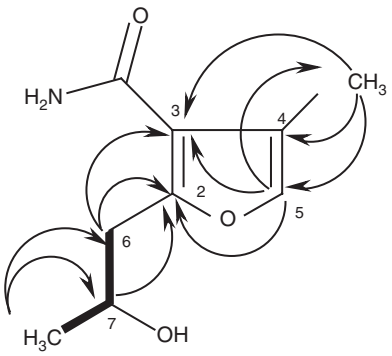

Fig. 4: HMBC (→) and H, H COSY (—) correlation of 2-(2-hydroxy-propyl)-4-methylfuran-3-carboxylic acid amide (3).

In the HMBC spectrum, the sp^2 methine ($\delta_H = 7.09/\delta_C = 138.4$ ppm) and the propyl protons CH_2-6, and CH-7 all coupled with the low-field carbon at $\delta = 155.9$ ppm; consequently, the latter signal was assigned as C-2, and the methine as C-5 of the furan ring (Fig. 4). Among the atoms seen by 4-CH_3, only that at $\delta = 118.6$ ppm showed also a cross signal with CH_2-6. The resulting structure **3** of 2-(2-hydroxy-propyl)-4-methylfuran-3-carboxamide was confirmed unequivocally by further HMBC correlations (Fig. 4).

In contrast to compounds **1** and **2**, the furan **3** gave a CD spectrum (Fig. 5) of suitable quality to determine the absolute configuration. As for previous natural products [13], CD and ORD data were calculated with DFT methods using Spartan'14 [14] and Gaussian 09 [15] (see Section 3). The CD spectrum obtained for (*R*)-**3** (Fig. 5) agreed sufficiently well with the experimental data. This result was confirmed by the optical rotation, which delivered a Boltzmann-weighted value of − 35° at 589 nm, while − 42° was measured in methanol (Table 1).

For an additional control of the *ab initio* results, we also synthesized the Mosher derivatives (see Table S1 in the Supporting Information) [12]. The ^{1}H NMR shift differences

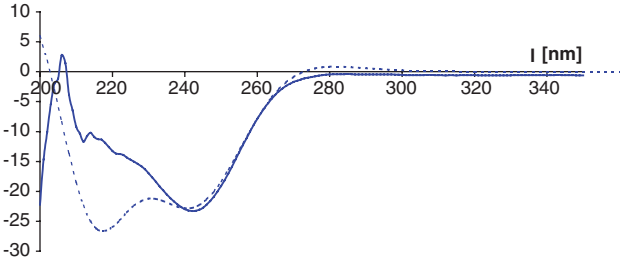

Fig. 5: Experimental (bold line, MeOH) CD spectrum of 2-(2-hydroxy-propyl)-4-methylfuran-3-carboxylic acid amide (**3**), and CD data calculated for (*R*)-**3** (dotted line).

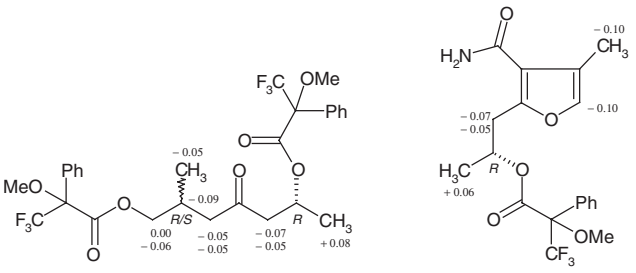

Fig. 6: NMR shift differences $\Delta\delta_{(S-R)}$ of the MTPA esters of **1** and **3**; the data were measured in $CDCl_3$ at 600 MHz. Due to the mixture of diastereomers, for some atoms of **1**, two differences were obtained.

between the (*S*)- and (*R*)-MTPA esters resulted in a positive $\Delta\delta_{(S-R)}$ value for the methyl protons at C-8, and negative values for the remaining protons on the other side of the molecule (Fig. 6). All the results above assigned the configuration of **3** unequivocally as (7*R*).

Furan containing compounds (e.g. albafuran A and roseophilin) are widespread in nature and are exhibiting diverse biological activities [1, 2, 16–20]. The physicochemical properties of the new compounds (**1–3**) are listed in Table 1.

Three further compounds, 4-vinylanisole (**4a**), phenethyl alcohol [21] and *p*-vinylphenol (**4b**) [22, 23] were identified by NMR and MS, and by comparison with literature data; **4a** was previously obtained by synthesis, but is a new natural product. GC-MS analysis of the nonpolar extract of the mycelia indicated further the presence of tetradecanoic acid, hexadecanoic acid, 9-octadecenoic acid, 2-methoxy-4-vinylphenol (**4c**) [24], 4-hydroxy-3-methoxybenzaldehyde, *o*-hydroxybiphenyl, 1,5,9-trimethyl-4,8,13-cyclotetradecatrien-1,3-diol (**5**, tentatively), and 1,1′-(*p*-chloro)-bisphenyl sulfone, a widespread pollutant (Table 3).

The crude extract and the isolated compounds **1–3** were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Rhizoctonia solani* and *Pythium ultimum*, using the agar diffusion method with paper disks (40 µg per disc). No activity was found for the extract and the isolated metabolites. Compounds (**1–3**) exhibited, however, weak brine shrimp toxicity (3%–8.3%, 10 µg mL^{-1}, Table 4).

3 Experimental section

3.1 General experimental procedure

The NMR spectra were measured on a Bruker AMX 300 (300.135 MHz) (Bruker Daltonics, Bremen, Germany), a Varian Unity 300 (300.145 MHz) and a Varian Inova 600 (150.820 MHz) spectrometer (Varian Deutschland GmbH, Darmstadt, Germany). DCI-MS: Finnigan MAT 95 A, 200 eV, Reactant gas NH_3. Optical rotation was measured on a Perkin–Elmer polarimeter, model 241. UV/Vis spectra were recorded on a Perkin–Elmer Lambda 15 UV/VIS spectrometer (Billerica, MA, USA). ESI-MS was recorded on a MicrOTOF (Bruker Daltonik, Bremen, Germany) with quaternary pump Rheos 4000 (Flux Instrument). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (Thermo Electron, Inc., San Jose, CA, USA) (70 eV) with perfluorokerosene as reference substance for EI HRMS. GC-MS was measured on a Trace GC-MS (Thermo Finnigan, San Jose, CA, USA), ionization mode EI eV 70, instrument equipped with a capillary column CP-Sil 8 CB for amines (length 30 m; inside diameter: 0.25 mm; outside diameter: 0.35 mm; film thickness: 0.25 µm). The analysis was carried out at a programmed temperature: initial temperature 40°C (kept for 1 min), then increasing at a rate of 10°C min^{-1} and final temp 280°C (kept for 10 min), injector temp was 250°C and detector (mode of ionization: EI) temp at

Table 3: GC-EI-MS analysis for the identification of nonpolar metabolites; for the conditions see the experimental part.

Name	R_t (min)	Relative abundance (%)	Mol. formula	Mol. weight
2-Methoxy-4-vinylphenol (**4c**)	12.51	20	$C_{18}H_{36}O$	268
4-Hydroxy-3-methoxy benzaldehyde	13.70	55	$C_{17}H_{34}$	238
o-Hydroxybiphenyl	15.28	75	$C_{12}H_{10}O$	170
Tetradecanoic acid	17.49	30	$C_{14}H_{28}O_2$	228
9-Octadecanoic acid	18.71	97	$C_{18}H_{34}O_2$	282
Hexadecanoic acid	19.64	100	$C_{16}H_{32}O_2$	256
p-Vinylphenol (**4b**)	21.02	38	C_8H_8O	120
1,5,9-Trimethyl-4,8,13-cyclotetradecatrien-1,3-diol (**5**)	22.57	43	$C_{20}H_{34}O_2$	306
1,1′-(*p*-Chloro)-bisphenyl sulphone	22.91	100	$C_{12}H_{22}O_2S$	268

Table 4: Cytotoxic activity of compounds **1–3** in the brine shrimp microwell test at 10 μg mL^{-1}.

Compounds	Lethality (%) of brine shrimps
Crude extract	11.2
1	8.3
2	1.5
3	3.0
Actinomycin D	100

250°C, He as a carrier gas at flow rate 1 mL min^{-1}, total run time 27 min and injection volume 0.2 μL. Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel, Düren, Germany). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany), XAD-16 (Dow Chemical Company; purchased from Sigma-Aldrich Chemie, Steinheim, Germany) resin was obtained from Rohm and Haas.

3.2 Isolation and taxonomy of the producing strain

The terrestrial *Streptomyces* strain ANK245 has been derived from a soil sample and was isolated at room temperature on YMG agar (2 g L^{-1} yeast extract, 5 g L^{-1} malt extract, 5 g L^{-1} glucose, 15 g L^{-1} agar, 30 mg L^{-1} cycloheximide). The strain is deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany. Its almost complete 16S rRNA gene sequence is available in GenBank (Accession Nr. HQ662223). The sequence shows the highest homology to *Streptomyces drozdowiczii* strain NBRC101007 (GenBank Accession Nr. AB249957) with 99.6%. Similar homologies were found to *Streptomyces sanglieri* strain NBRC100784 (GenBank accession Nr. AB249945) with 99.2%, and to *Streptomyces atratus* strain NRRLB-16927 (GenBank Accession Nr. DQ026638) with 99.1%, which share high sequence similarities to the *Streptomyces griseus* clade (Rong & Huang, 2010) [25].

3.3 Fermentation, extraction, and isolation

From an agar sub-culture, the strain was primarily cultivated as 3 L culture using M_2 medium on a shaker for 7 days at 28°C. The culture broth was then used to inoculate a 50 L fermenter, using the same conditions as above, for additional 7 days. After harvesting, the broth was mixed with Celite (~2 kg) and filtered off. The mycelial cake was extracted with ethyl acetate (3×) followed by acetone (1×). The acetone extract was concentrated *in vacuo*, and the remaining water residue was re-extracted with acetyl acetate. The water phase was extracted by filtration over an XAD-16 column (120×8 cm), and the adsorbed organic material was eluted with methanol and the extract concentrated *in vacuo* at 40°C to the aqueous residue. The remainder was re-extracted by ethyl acetate. The ethyl acetate extracts from filtrate and mycelium were separately evaporated to dryness, and afforded 3.6 g and 4.3 g, respectively, as dark brown oils. The mycelial extract (4.3 g) consisted mostly of fatty acids, and by GC-MS analysis the components listed in Table 3 were identified.

The filtrate extract (3.6 g) was fractionated on a silica gel column by elution with a CH_2Cl_2–MeOH gradient. Under TLC control, four fractions; I (0.3 g), II (0.45 g), III (0.5 g) and IV (0.25 g) were obtained. The first fraction I was re-fractionated on silica gel (CH_2Cl_2–MeOH), followed by purification on Sephadex LH-20 (column, CH_2Cl_2-40% MeOH) to afford phenethyl alcohol (1.2 mg), *p*-vinylanisole (**4a**, 1.0 mg) and *p*-vinylphenol (**4b**, 1.0 mg) as colorless oils. Purification of fraction III using silica gel column (CH_2Cl_2–MeOH) gave two components. The first of them was further purified by Sephadex LH-20 (MeOH) to afford a colorless solid of 2-(2-hydroxypropyl)-4-methylfuran-3-carboxylic acid amide (**3**, 7.5 mg), while the other one was purified by Sephadex LH-20 (DCM-40% MeOH), giving a colorless oil of 1,6-dihydroxy-2-methyl-heptan-4-one (**1**, 12.8 mg). Finally, purification of fraction IV using a silica gel column (DCM-MeOH), followed by Sephadex LH-20 (MeOH) yielded 4-hydroxy-1-(2-methyl-oxiranyl)-pentan-2-one (**2**) as yellow oil (15.6 mg). For data of **1**–**3** see Tables 1 and 2.

***p*-Vinylanisole (4a)**: $C_9H_{10}O$ (134); colorless oil, turned purple with anisaldehyde-sulfuric acid, $R_f = 0.51$ (CH_2Cl_2). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.90$ (m, 4 H, Ar-H), 6.64 (dd, $^3J = 17.5$, 10.8 Hz, 1 H, 1′-H), 5.59 (dd, $^3J = 17.5$, $^2J = 0.9$ Hz, 1 H, 2′-H_a), 5.12 (dd, $^3J = 10.8$, $^2J = 0.9$ Hz, 1 H, 2′-H_b), 3.92 (s, 3H, 1-OCH_3).

***p*-Vinylphenol (4b)**: C_8H_8O (120); colorless oil, turned pink with anisaldehyde-sulfuric acid, $R_f = 0.34$ (CH_2Cl_2). – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.31$ (d, $^3J = 8.7$ Hz, 2 H, 3/5-H), 6.79 (d, $^3J = 8.7$ Hz, 2 H, 2/6-H), 6.65 (dd, $^3J = 17.6$, 10.7 Hz, 1H, 1′-H), 5.60 (dd, $^3J = 17.6$, $^2J = 1.0$ Hz, 1 H, 2′-H_a), 5.12 (dd, $^3J = 10.9$, $^2J = 1.0$ Hz, 1H, 2′-H_b).

3.4 Antimicrobial activity

Antimicrobial assays were conducted utilizing the disc-agar method [26] against diverse sets of microorganisms.

The newly isolated compounds (**1–3**) were dissolved in CH_2Cl_2–10% MeOH at a concentration of 1 mg mL^{-1}. Aliquots of 40 µL were soaked on filter paper disks (9 mm ∅, no. 2668, Schleicher & Schüll, Germany) and dried for 1 h at room temperature under sterilized conditions. The paper disks were placed on inoculated agar plats and incubated for 24 h at 38°C in the dark (bacteria and fungi) or at room temperature in sunlight (algae).

3.5 Brine shrimp microwell cytotoxicity assay

The cytotoxic assay was performed according to Takahashi et al. [27] and Sajid et al. [28]. Actinomycin D (10 µg mL^{-1}) was used as positive control, and DMSO (10 µL) was used as blind value.

3.6 Quantum-mechanical calculations

The conformer distribution of **1** and **3** was determined with Spartan'14 [14], using the Merck Molecular Force Field (MMFF). All conformers with energies <7 kcal mol^{-1} above the global minimum were further optimized with Spartan'14 using DFT calculations with the WB97XD functional and the 6-31G* basis set. The resulting geometries were used to calculate with Gaussian 09 [15] the Boltzmann factors [basis set 6-311+G(2df, 2p)], the CD data [rb3lyp/6-311 g(2d, p)], and the optical rotations [WB97XD/6-311 g(d, p)]. The CD (Fig. 5) and ORD data were weighted according to their Boltzmann factors. For the optical rotation of (2*S*, 6*R*)-**1**, a value of −164° was calculated, while for (2*R*, 6*R*)-**1**, +71° were obtained; for an estimated 1 : 1 mixture of the diastereomers, a rotation of −93° resulted.

4 Supporting information

NMR spectra and other supplementary data associated with this article are given as supporting information available online (DOI: 10.1515/znb-2016-0202).

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