The Journal of Antibiotics (2011) 64, 205–209
© 2011 Japan Antibiotics Research Association All rights reserved 0021-8820/11 $32.00
www.nature.com/ja

NOTE

Glucopiericidin C: a cytotoxic piericidin glucoside antibiotic produced by a marine *Streptomyces* isolate*

Khaled A Shaaban[1], Elisabeth Helmke[2], Gerhard Kelter[3], Heinz Herbert Fiebig[3] and Hartmut Laatsch[1]

The Journal of Antibiotics (2011) **64,** 205–209; doi:10.1038/ja.2010.125; published online 17 November 2010

Keywords: cytotoxic antibiotic; marine *Streptomyces*; piericidin

More than 35 naturally occurring piericidins[1,2] and closely related antibiotics[3,4] have been reported. They are derived from prenylated polyoxypyridines and exhibit interesting biological activities. Among them, glucopiericidin A (**1**), B and some others display antimicrobial properties and *in vitro* inhibitory activity against antibody formation.[5] For glucopiericidiols A1 and A2, additional cytocidal activities against HeLa S3 cells *in vitro* have been reported,[6] and others demonstrate insecticidal activity.[7] The biological activities of the glucopiericidins are strongly influenced by the structure and position of the sugar unit: glucopiericidins A (**1**) and B show higher antimicrobial activities than piericidin A (**2**) or other glycosides such as 3′-rhamnopiericidin A1 (SN-198-C),[8] 3′-deoxytalopiericidin A1 (DTPA)[9] and 7-demethyl-3′-rhamnopiericidin A1.[10] In contrast, acute toxicities of the latter three substances in mice were lower than those of piericidin A (**2**).[5] In our search for new secondary metabolites from marine bacteria, we isolated a new cytotoxic piericidin derivative, glucopiericidin C (**3**), and for the first time as natural product, 5-oxo-5-*o*-tolyl-pentanoic acid (**8a**) together with further 10 known metabolites. The algal metabolite spatozoate (**6**) was isolated here for the first time from bacteria.

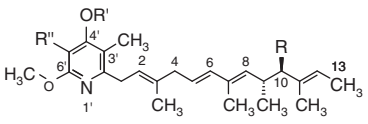
The crude extract obtained by fermentation of the marine-derived *Streptomyces* isolate B8112 was moderately active against Gram-positive and Gram-negative bacteria and *Candida albicans*, but strongly active against the fungus *Mucor miehei* (Tü284). Additionally, a pronounced *in vitro* antitumor activity with a mean IC_{70} of $<10\,\mu g\,ml^{-1}$ was found in a six cell line panel.[11] TLC of the extract exhibited numerous UV absorbing bands: two of them (**3**, **6**) turned blue with anisaldehyde/sulfuric acid and later to dark green in the case of **3**, whereas **8a** changed to purple on standing.

For preparative isolation, the strain was cultivated on M_2^+ medium as a shake culture at 28 °C for 7 days, and extracted as reported previously.[12] Chromatography on Sephadex LH-20 (Lipophilic Sephadex, Amersham Bioscience, purchased from Sigma-Aldrich Chemie, Steinheim, Germany) led to the isolation of indole-3-carboxylic acid and uracil; flash silica gel column chromatography of fraction I delivered five subfractions (SFs) A∼E. Purification of the less polar SF A led to spatozoate (**6**). Further purification of SF C on Sephadex LH-20 gave 5-(2-methylphenyl)-4-pentenoic acid) (**7**)[13] and monensin B,[14,15] whereas the middle polar SF D afforded 5-oxo-5-*o*-tolyl-pentanoic acid (**8a**), along with glucopiericidin A (**1**),[6] piericidin A (**2**)[5,16] and phenylacetic acid. Separation of the most polar SF E by PTLC, followed by chromatography on Sephadex LH-20, gave glucopiericidin C (**3**), 2′-deoxy-uridin and 2′-deoxy-thymidin. The known compounds were identified by means of their NMR and mass data using AntiBase,[2] and by comparison with authentic spectra.

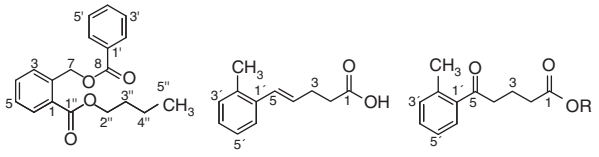
The physicochemical properties of compound **3** are summarized in Table 1. The UV data and the proton NMR spectrum (Supplementary Figure F2) indicated a close similarity with glucopiericidin A (**1**). However, the 4′-methoxy signal of **1** at δ 3.85 was replaced by a proton s at δ 5.86 (δ_C 92.8, Table 2). The loss of the methoxy group was also confirmed by the difference in the molecular formula $C_{30}H_{45}NO_8$ of glucopiericidin C (**3**), determined by ESI-HRMS (Supplementary Figure S5).

From HMBC and H, H COSY correlations of **3** (Figure 1 and Supplementary Figure S4), two partial structures were obtained: the 5′-demethoxypiericidin A aglycone **5** (also known as Mer-A2026-A[3]) and a hexose system. Further evidence was obtained from the ESI-MS^2 spectra, which delivered a fragment ion at *m/z* 368 because of the loss of the sugar moiety ($C_6H_{11}O_6$) from the parent compound **3**. The two possibilities for connection of the sugar system are position C-4′ of the pyridine system (as in **4**) or position C-10 of the aglycone. As was observed for **4**, an HMBC coupling of the acetal proton with the pyridine ring would be expected if the sugar system was attached at position C-4′ of the pyridine system. In this case, however, the HMBC experiments showed clear cross signals between the anomeric proton (δ 4.20) and C-10 (δ 93.7), and conversely a coupling from H-10

[1]Institute of Organic and Biomolecular Chemistry, University of Göttingen, Göttingen, Germany; [2]Alfred Wegener Institute of Polar and Marine Research, Bremerhaven, Germany and [3]Oncotest GmbH, Freiburg, Germany
Correspondence: Professor H Laatsch, Institute of Organic and Biomolecular Chemistry, Georg-August University, Tammannstrasse 2, Goettingen D-37077, Germany.
E-mail: hlaatsc@gwdg.de
*Article no. 43 on 'Marine Bacteria'. Article no. 42: *C. B. Fotso Fondja Yao, W. Al Zereini, S. Fotso, H. Anke, H. Laatsch*: Aqabamycins A-G: Novel Nitro Maleimides from a Marine *Vibrio* species. *J. Antibiot.* **63**, 303–308 (2010).
Received 6 July 2010; revised 14 September 2010; accepted 26 September 2010; published online 17 November 2010



1: R = A, R' = H, R'' = OCH_3
2: R = OH, R' = H, R'' = OCH_3
3: R = A, R' = R'' = H
4: R = R'' = H, R' = A
5: R = R' = R'' = H



6 **7**

8a: R = H
8b: R = CH_3
8c: R = H, 2'-NH_2 instead of 2'-CH_3

Table 1 Physicochemical properties of glucopiericidin C (3)

Appearance	*Yellow oil*
R_f (silica gel)	0.19 (CH_2Cl_2/10% MeOH)
Solubility	Soluble in DMSO, MeOH, EtOH and EtOAc, insoluble in hexane, benzene and H_2O.
Molecular formula	$C_{30}H_{45}NO_8$
(+)-ESI-MS: *m/z* (%)	1117 ([2M+Na]$^+$, 100), 570 ([M+Na]$^+$, 78), 548 ([M+H]$^+$, 12)
(−)-ESI-MS: *m/z* (%)	1093 ([2M-H]$^-$, 100), 546 ([M-H]$^-$, 82)
(+)-HRESI-MS (*m/z*)	Found 548.321770, calcd. 548.32179 for $C_{30}H_{46}NO_8$ ([M+H]$^+$)
Optical rotation	$[\alpha]_D^{20}$ -13° (*c*. 0.2, MeOH)
UV (MeOH): λ_{max}(log ε)	(MeOH): 223 (4.26), 239 (4.30); (MeOH/HCl): 204 (4.36), 235 (4.27), 271 (3.74); (MeOH/NaOH): 209 (4.37), 238 (4.36), 270 (3.38) nm

(δ 3.71) towards the anomeric carbon C-1″ (δ 104.1), pointing to a connection of the sugar moiety with C-10. This was further confirmed by the ^{13}C NMR shift of C-10, in which a value of δ 93.7 was observed, similar to that observed for **1**, whereas the corresponding methine carbon signal of piericidin A (**2**), with a free OH group at C-10, is found at δ 82.8 (Supplementary Figure S3).

Compound **6** was obtained as a UV absorbing colorless oil of medium polarity, which turned blue with anisaldehyde/sulfuric acid. A database search in AntiBase[2] with the molecular formula $C_{19}H_{20}O_4$ (deduced by EI-HRMS) delivered only two hits, ochracenomicin B and spatozoate (**6**). The structure of the latter one was fully confirmed by the literature values.[18] It is reported here for the first time as a bacterial metabolite, but had been obtained previously from the brown alga *Spatoglossum variabile*,[17] and might be formed there under the influence of associated or endophytic bacteria.

The colorless semisolid compound **8a** stained purple with anisaldehyde/sulfuric acid and could be separated from contaminating phenylacetic acid only after methylation with diazomethane. Its molecular formula was established by ESI-HRMS as $C_{12}H_{14}O_3$. The ^{1}H NMR spectrum displayed a narrow multiplet of three adjacent benzene protons between δ 7.20∼7.37 and additionally an *o/m*-coupled 1H dd signal at δ 7.61, suggesting a carbonyl group in the *o*-position of the respective proton. Three methylene groups in the aliphatic region indicated a 1, 3-disubstituted propanediyl system. A 3H s at δ 2.47 indicated a Me group attached to the aromatic system, which was confirmed by HMBC correlations into the ring.

In the ^{13}C NMR spectrum of **8a**, two carbonyls at δ 203.4 and δ 179.6 indicated the presence of a conjugated ketone and a carboxylic acid, respectively. Owing to the shift of H-6′, it was deduced that the ketone must be attached directly to the benzene ring. As only H-6′ showed an HMBC correlation with the ketone signal, and had an unusual downfield shift, it was established that the Me group must be located on the other *o*-position, at C-2′. The middle methylene group of the propanediyl system at C-3 was coupled with both carbonyls, and coupling was observed between the methylene at C-2 and the C-1 carbonyl, and between the methylene at C-4 and the carbonyl at C-5. The NMR data of the Me ester **8b** confirmed the structure of the parent compound as 5-oxo-5-*o*-tolylpentanoic acid (**8a**, Figure 2), which had been obtained previously by synthesis; spectroscopic data were not reported.[19] Remarkably, 5-phenylpentanoic acids are very rare in nature. Beside **7**,[12] one of the closest relatives is ovalic acid (**8c**) from *Pseudomonas ovalis*.[20]

Antibacterial and antifungal activities were qualitatively determined using the agar diffusion method.[21] Glucopiericidin C (**3**) displayed the same antibacterial activity as glucopiericidin A (**1**) against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative *Escherichia coli*. In addition, it showed activity against *Chlorella vulgaris* and other microalgae. Glucopiericidin C (**3**) also displayed a concentration-dependent cytotoxicity towards a panel of 36 human tumor cell lines,[11] with a mean IC_{50} of 2.0 μM (mean IC_{70}=4.2 μM). Especially prostate cancer-derived cell lines (all the four) as well as lung, mammary, kidney and uterus cell lines showed a significant sensitivity.[11] An IC_{70} based Compare Analysis with 100 reference compounds delivered no significant correlation, which may indicate a new or unknown mode of action. A similar biological profile was observed for 5-*o*-tolyl-4-pentenoic acid.

EXPERIMENTAL PROCEDURE

Materials and methods

NMR spectra were measured on Varian Unity 300 (^{1}H, 300. 145 MHz) spectrometer (Varian Deutschland GmbH, Darmstadt, Germany). ESI-HRMS was carried out on a Finnigan LCQ ion-trap MS (Fisher Scientific GmbH, Schwerte, Germany) coupled with a Flux Instruments (Basel, Switzerland) Rheos 4000 quaternary pump and an HP 1100 HPLC (nucleosil column, EC 125/2, 100–5, C 18) with a diode array detector (Finnigan Surveyor LC System). HR-MS were recorded by ESI MS on an Apex IV 7 Tesla FT Ion Cyclotron Resonance MS (Bruker Daltonics, Billerica, MA, USA). EI-MS were recorded on a Finnigan MAT 95 spectrometer (70 eV, Thermo Electron, Bremen, Germany) using perfluorokerosene as the reference substance for EI-HRMS. UV/vis spectra were recorded on a Varian Cary 3E UV/vis spectrometer (Varian Deutschland GmbH). Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on silica gel TLC cards Polygram SIL G/UV_{254} (Macherey-Nagel & Co, 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 resin was obtained from Rohm and Haas (Frankfurt, Germany).

Taxonomy of the producing strain

The actinomycete strain B8112 was isolated from sediment of the Laguna de Terminos in the Gulf of Mexico, using modified M3 agar medium[22] containing 50% natural seawater. The reference culture is kept on yeast extract-malt

Table 2 ^{13}C and ^{1}H NMR data of glucopiericidin C (3), glucopiericidin A (1) and piericidin A (2) (*J* in [Hz])

	*Glucopiericidin A (**1**)*		*Piericidin A (**2**)*	*Glucopiericidin C (**3**)*[a]	
Position	δ_C [b,c]	δ_H *(300 MHz)*[b]	δ_C *(75 MHz)*[b]	δ_C *(75 MHz)*[d]	δ_H *(300 MHz)*[d]
1	34.2	3.36 (d, 7.0)	34.3	32.2	3.32 (m)
2	121.9	5.60 (m)	122.1	121.0	5.20 (t, 7.0)
3	134.7	—	135.4	138.2	—
3-CH_3	16.6	1.73 (s)	13.1	16.7	1.72 (s)
4	42.9	2.77 (d, 7.0)	43.0	43.8	2.76 (m)
5	126.7	5.61 (dt, 15.5, 7.0)	126.7	125.9	5.51 (m)
6	135.5	6.05 (d, 15.5)	136.0	137.8	6.10 (d, 15.5)
7	134.6	—	134.7	134.5	—
7-CH_3	13.0	1.78 (s)	16.6	13.2	1.72 (s)
8	134.4	5.23 (d, 9.5)	133.0	136.2	5.38 (d, 9.1)
9	35.1	2.77 (m)	36.8	36.7	2.76 (m)
9-CH_3	16.9	0.75 (d, 7.0)	17.3	17.7	0.81 (d, 6.8)
10	94.4	3.45 (d, 4.0)	82.8	93.7	3.71 (d, 4.0)
11	135.3	—	135.6	136.9	—
11-CH_3	10.9	1.61 (s)	10.5	11.8	1.60 (s)
12	123.5	5.40 (m)	123.5	124.3	5.45 (m)
13	13.2	1.62 (d, 6.0)	13.1	13.1	1.60 (brs)
2′	150.6	—	150.8	149.3[e]	—
3′	112.5	—	112.1	117.6[e]	—
3′-CH_3	10.5	2.08 (s)	10.4	10.4	1.95 (s)
4′	153.6	—	154.0	149.3[e]	—
5′	128.1	—	127.8	92.8	5.86 (s)
5′-OCH_3	60.6	3.85 (s)	53.0	—	—
6′	154.5	—	153.6	161.7	—
6′-OCH_3	53.6	3.94 (s)	60.5	56.1	3.84 (s)
1″	103.7	4.14 (d, 8.0)	—	104.1	4.20 (d, 7.8)
2″	74.3	3.23 (t, 8.0)	—	75.7	3.14 (m)
3″	76.3	3.48 (t, 8.0)	—	78.2	3.32 (m)
4″	70.7	3.42 (m)	—	71.5	3.28 (m)
5″	75.3	3.28 (m)	—	77.7	3.10 (m)
6″	62.7	3.64 (dd, 12.0, 6.0)			
		3.81 (brd, 12.0)	—	62.7	3.72, 3.62 (*ABX*, 11.8, 5.0, 2.6)

[a]See also Figures S2–S5.
[b]$CDCl_3$.
[c]125 MHz.
[d]CD_3OD.
[e]Weak signal, shift taken from HMBC spectrum.

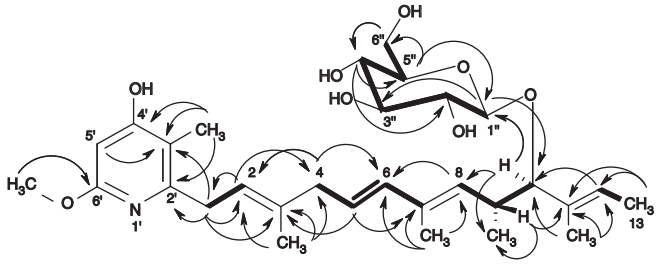


Figure 1 Selected HMBC couplings (→) and H, H COSY correlations (bold lines) of glucopiericidin C (**3**).

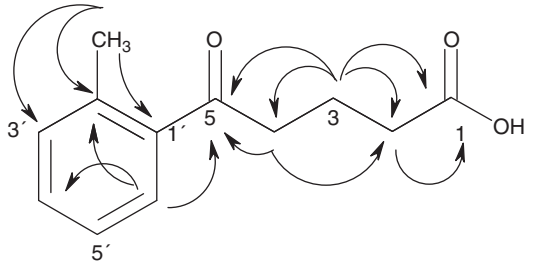


Figure 2 (→) HMBC connectivities of 5-oxo-5-*o*-tolyl-pentanoic acid (**8a**).

extract-agar[23] in the collection of marine actinomycetes at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven.

The almost complete 16S rRNA gene sequence of B8112 was identical (similarity=100%) to that of the *Streptomyces griseoaurantiacus* type strain DSM 40430. The strain formed gray aerial mycelia with spiral spore chains (*Spirales*). Spores were cylindrical, 0.5–0.7 μm in diameter and about 1.1 μm in length, with a smooth surface (Supplementary Figure S1). The vegetative mycelium was strawberry red on yeast extract-malt extract-agar, and diffusible pigment was not formed. Growth temperatures were tested in a yeast extract-malt extract-medium. The optimal growth temperature was about 30 °C.

Growth was not obtained at 4 and 46 °C, and salinity tolerance was low. The strain developed only in the range of 0–3% (w/v) NaCl, not with 7, 10 or 13% NaCl, in yeast extract-malt extract-medium. Melanin pigment was neither produced on peptone-yeast extract-iron agar nor on tyrosine agar.[24] Chitin was not cleaved, but starch was degraded.[25] The strain was catalase positive, and nitrate reductase was formed.

Cytotoxicity tests

A modified propidium iodide assay was used to assess the compounds' cytotoxic activity towards a panel of 36 human tumor cell lines.[26] A total of 24 cell lines were derived from patient tumors engrafted as a s.c. growing tumor in NMRI nu/nu mice. The other cell lines were obtained from American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA or DSMZ (German Collection of Microorganisms, Braunschweig, Germany).

M_2^+ medium

A solution of glucose (4 g), yeast extract (4 g) and malt extract (10 g) in 0.5 l of artificial sea water and 0.5 l of tap water was set to pH 7.8 with 2N NaOH and sterilized for 30 min at 121 °C.

Fermentation, extraction and isolation

The marine isolate B8112 was precultivated on M_2^+ agar plates (with 50% sea water) at 28 °C for 3 days. Pieces of a well-grown agar subculture were used to inoculate 175 of 1 l Erlenmeyer flasks each with 200 ml M_2^+ medium. After 7 days at 28 °C, a yellow-brown culture broth was obtained, which was filtered over Celite with the aid of a filter press. The filtrate was adsorbed on Amberlite XAD-16, and subsequently eluted with MeOH. The MeOH soln was concd, and the resulting aq. residue was extracted with Et acetate. The mycelial phase was extracted with acetone, the soln was concd *in vacuo* and the resultant aq. residue was extracted with Et acetate. The two extracts were similar on TLC and were combined. Evaporation gave 5.8 g of a brown residue.

Separation of the extract on Sephadex LH-20 (column 3×60 cm, CH_2Cl_2/50% MeOH) delivered two fractions, I (0.35 g) and II (3.6 g). Further chromatography of fraction I on a flash silica gel column (CH_2Cl_2-MeOH gradient) gave five SFs: A (0.37 g), B (0.30 g), C (0.25 g), D (0.95 g) and E (0.41 g). Purification of SF A by PTLC on silica gel, followed by Sephadex LH-20, led to spatozoate (**6**, 16.3 mg). Chromatography of SF C on Sephadex LH-20 (MeOH) gave 5-*o*-tolyl-4-pentenoic acid (**7**, 53.3 mg) and monensin B (53.7 mg), whereas SF D afforded 5-oxo-5-*o*-tolyl-pentanoic acid (**8a**, 16.3 mg), piericidin A (**2**, 120.7 mg), glucopiericidin A (**1**, 35.1 mg) and phenylacetic acid (10.3 mg). PTLC of the polar SF E, followed by chromatography on Sephadex LH-20, afforded glucopiericidin C (**3**, 22.4 mg), along with 2′-deoxy-uridin (6.3 mg) and 2′-deoxy-thymidin (11.3 mg). Purification of fraction II on silica gel led to indole-3-carboxylic acid (10.3 mg) and uracil (15.3 mg).

Spatozoate (6)

Colorless oil, UV absorbing, blue on TLC using anisaldehyde/sulfuric acid, R_f 0.41 (cyclohexane/10% EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (dd, 7.5, 1.5 Hz, 1H, H-6), 7.72 (m, 1H, H-3), 7.54 (td, 7.5, 1.5 Hz, 1 H, H-5), 7.52 (td, 7.5, 1.5 Hz, 1H, H-4), 7.46 (dd, 8.3, 1.8 Hz, 2H, H-2′), 7.38 (td, 8.3, 1.8 Hz, 2H, H-3′), 7.30 (dd, 8.5, 1.8 Hz, 1H, H-4), 5.36 (s, 2H, H-7), 4.10 (t, 7.0 Hz, 2H, H-2″) 1.39 and 1.65 (4H, m, H-4″/3″), 0.92 (t, 7.4 Hz, 3H, H-5″). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5 (C-8), 167.2 (C-I″), 135.3 (C-2), 132.3 (C-1), 131.6 (C-1′, 131.0 (C-6), 130.8 (C-3), 128.8 (C-5), 128.7 (C-4), 128.4 (C-2″), 128.2 (C-3′), 128.2 (C-4′, 67.2 (C-7), 65.4 (C-2″), 30.3 (C-3″), 19.0 (C-4″) and 13.6 (C-5″). EI MS (70 eV): *m/z* (%) 312 ($[M]^{+\cdot}$, 10), 206 ($[M-(PhCO)]^{+\cdot}$, 34), 149 (100) and 91 (50).

5-Oxo-5-*o*-tolyl-pentanoic acid (8a)

Colorless wax-like solid, UV absorbing, purple on TLC using anisaldehyde/sulfuric acid, R_f =0.18. (CH_2Cl_2/3% MeOH). ^{1}H and ^{13}C NMR (300/50 MHz, $CDCl_3$) (see Table 3). DCI MS (NH_3) *m/z* (%) 430 ($[2M+NH4]^+$, 8), 224 ($[M+NH_4]^+$, 100), 207 ($[M+H]^+$, 16). (+)-ESI-HRMS: *m/z* 229.08352 (calcd 229.08352 for $C_{12}H_{14}O_3Na$).

Table 3 ^{13}C and ^{1}H NMR data of 5-*o*-tolyl-4-pentenoic acid[12] (7) and 5-oxo-5-*o*-tolyl-pentanoic acid (8a) in $CDCl_3$ (coupling constants *J* in [Hz])

	*5-o-Tolyl-4-pentenoic acid (**7**)*		*5-Oxo-5-o-tolyl-pentanoic acid (**8a**)*	
Position	δ_C [a]	δ_H [b]	δ_C [c]	δ_H [b]
1	179.2	—	179.6	—
1-OH	—	—	—	10.54 (brs)
2	28.2	2.58 (m)	33.0	2.44 (t, 7.1)
3	33.9	2.58 (m)	18.9	2.01 (m)
4	129.2	6.08 (dm, 15.7)	39.9	2.95 (t, 7.2)
5	139.3	6.65 (d, 15.8)	203.4	—
1′	135.1	—	137.1	—
2′	136.4	—	138.1	—
2′-CH_3	19.8	2.32 (s)	21.0	2.47 (s)
3′	130.2	7.13 (m)	132.0	7.30 (m)
4′	127.1	7.13 (m)	131.4	7.30 (m)
5′	126.0	7.13 (m)	125.7	7.30 (m)
6′	125.5	7.39 (m)	128.5	7.61 (dd, 8.3, 1.2)

[a]75 MHz.
[b]300 MHz.
[c]50 MHz.

5-Oxo-5-*o*-tolyl-pentanoic acid Me ester (8b)

Colorless oil, obtained by methylation of 5-oxo-5-*o*-tolyl-pentanoic acid (**8a**) with diazomethane. UV absorbing, purple on TLC with anisaldehyde/sulfuric acid, R_f =0.78 (CH_2Cl_2/4% MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dd, 8.3, 1.2 Hz, 1 H, H-6′), 7.40-7.25 (m, 3 H, H-3′, H-4′ H-5′, 3.70 (s, 3H, 1-OCH_3), 2.96 (t, 7.2 Hz, 2H, CH_2-4), 2.50 (s, 3 H, 2′-CH_3), 2.44 (t, 7.1 Hz, 2H, CH_2-2), 2.05 (m, 2H, CH_2-2). EI MS (70 eV) *m/z* (%) 220 ($[M]^+$, 8), 189 (14), 161 (16), 119 (100), 91 (96), 65 (32).

ACKNOWLEDGEMENTS

We thank R Machinek for the NMR spectra, Dr H Frauendorf for the MS data, F Lissy for biological activity determinations and A. Kohl for technical assistance. This investigation was supported by the Ministry of Education and Research (BMBF, 03F0415A).

1 *Dictionary of Natural Products on CD-ROM* (CRC-Press, Boca Raton, Florida, USA, 2009).
2 Laatsch, H. *AntiBase 2010* A data base for rapid structural determination of microbial natural products, and annual updates,Wiley/VCH: Weinheim, Germany.seehttp://wwwuser.gwdg.de/~ucoc/laatsch/AntiBase.htm.
3 Kominato, K., Watanabe, Y., Hirano, S., Kioka, T., Terasawa, T., Yoshioka, T., Okamura, K. & Tone, H. Mer-A2026A and B, novel piericidins with vasodilating effect I producing organism, fermentation, isolation and biological properties. *J. Antibiot* **48,** 99–102 (1995).
4 Kominato, K., Watanabe, Y., Hirano, S., Kioka, T., Terasawa, T., Yoshioka, T., Okamura, K. & Tone, H. Mer-A2026A and B, novel piericidins with vasodilating effect. II. Physico-chemical properties and chemical structures. *J. Antibiot.* **48,** 103–105 (1995).
5 Matsumoto, M., Mogi, K., Nagaoka, K., Ishizeig, S., Kawahara, R. & Nakashiha, T. New piericidin glucosides, glucopiericidins A and B. *J. Antibiot.* **40,** 149–156 (1987).
6 Funayama, S., Ishibashi, M., Anraku, Y., Miyauchi, M., Mori, R., Komlyama, K. & Omura, S. Novel cytocidal antibiotics, glucopiericidinols A1 and A2. Taxonomy, fermentation, isolation, structure elucidation and biological characteristics. *J. Antibiot.* **42,** 1734–1740 (1989).
7 Takahashi, N., Suzuki, A., Kimura, Y., Miyamoto, S., Tamura, S., Mitsui, T. & Fukami, J. Isolation, structure, and physiological activities of piericidin B, natural insecticide produced by a *Streptomyces*. *Agric. Biol. Chem.* **32,** 1115–1122 (1968).
8 Kimura, K., Nakayama, S., Nakajima, N., Yoshihama, M., Miyata, N. & Kawnishi, G. A new piericidin rhamnoside, 3′-rhamnopiericidin A1. *J. Antibiot.* **43,** 1341–1343 (1990).
9 Iwaski, H., Kamisango, K. I., Kuboniwa, H., Sasaki, H. & Matsubara, S. 3′-Deoxytalo-piericidin A1, a novel analog of antitumor antibiotics fromoligotroph. *J. Antibiot.* **44,** 451–452 (1991).

10 Kimura, K. I., Takahashi, H., Myata, N., Yoshihana, M. & Uramoto, M. New piericidin antibiotics, 7-demethylpiericidin A1 and 7-demethyl-3'-rhamnopiericidin A1. *J. Antibiot.* **49,** 697–699 (1996).
11 Shaaban, K. A., Shaaban, M., Facey, P., Fotso, S., Helmke, E., Maier, A., Fiebig, H. H. & Laatsch, H. Electrospray ionization-mass spectra of piperazimycins A and B and γ-butyrolactones from a marine-derived *Streptomyces* sp. *J. Antibiot.* **61,** 736–746 (2008).
12 Bibani, M. A. F., Baake, M., Lovisetto, B., Laatsch, H., Helmke, E. & Weyland, H. Anthranilamides: new antimicrobial active substances from a marine *Streptomyces* sp. *J. Antibiot.* **51,** 333–340 (1998).
13 Mukku, V. J. R., Maskey, R. P., Monecke, P., Gruen-Wollny, I. & Laatsch, H. 5-(2-methylphenyl)-4-pentenoic acid from a terrestrial *Streptomycete*. *Z. Naturforsch.* **57B,** 335–337 (2002).
14 Reynolds, K. & Robinson, J. Biosynthesis of monensin. The intramolecular rearrangement of isobutyryl-CoA to n-butyryl-CoA. *J. Chem. Soc., Chem. Commun* **24,** 1831–1832 (1985).
15 Gani, D., O'Hagan, D., Reynolds, K. & Robinson, J. A. Biosynthesis of the polyether antibiotic monensin-A: stereochemical aspects of the incorporation and metabolism of isobutyrate. *J. Chem. Soc., Chem. Commun.* **14,** 1002–1004 (1985).
16 Schnermann, M. J. & Boger, D. L Total synthesis of piericidin A1 and B1. *J. Am. Chem. Soc.* **127,** 15704–15705 (2005).
17 Klyne, W. The configuration of the anomeric carbon atoms in some cardiac glycosides. *J. Biochem.* **47,** xli–ii (1950).
18 Rahman, A., Choudhary, M. I., Hayat, S., Khan, A. M., Ahmad, A. & Malik, S. Spatozoate and varninasterol from the brown alga *Spatoglossum variabile*. *Phytochemistry* **52,** 495–499 (1999).
19 Ghosal, M., Sinha, B. & Bagchi, P. Synthesis of the dicarboxylic acid C12H14O4-degradation product of picrotoxin. *J. Org. Chem.* **23,** 584–586 (1958).
20 Gieg, L. M., Otter, A. & Fedorak, P. M. Carbazole degradation by *Pseudomonas* sp. LD2: metabolic characteristics and the identification of some metabolites. *Environm. Sci. Technol.* **30,** 575–585 (1996).
21 Sajid, I., Shaaban, K. A., Frauendorf, H., Hasnain, S. & Laatsch, H. Val-geninthiocin: structure elucidation and MS^n fragmentation of thiopeptide antibiotics produced by *Streptomyces* sp. RSF18. *Z. Naturforsch.* **63,** 1223–1230 (2008).
22 Rowbotham, T. J. & Cross, T. Ecology of *Rhodococcus coprophilus* and associated actinomycetes in fresh water and agricultural habitats. *J. Gen. Microbiol.* **100,** 231–240 (1977).
23 Weyland, H. Distribution of actinomycetes on the sea floor (1981). *Zbl. Bakt. Suppl.* **11,** 185–193 (1981).
24 Shirling, E. B. & Gottlieb, D. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16,** 313–340 (1966).
25 Helmke, E. & Weyland, H. *Rhodococcus marinonascens* sp. nov., an actinomycete from the sea. *Int. J. Syst. Bacteriol.* **34,** 127–138 (1966).
26 Dengler, W. A., Schulte, J., Berger, D. P., Mertelsmann, R. & Fiebig, H. H. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. *Anticancer Drugs* **6,** 522–532 (1995).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)