Myxotyrosides A and B, Unusual Rhamnosides from *Myxococcus* sp.

Birgit Ohlendorf,[†] Wolfram Lorenzen,[‡] Stefan Kehraus,[†] Anja Krick,[†] Helge B. Bode,[‡] and Gabriele M. König*,[†]

Institute of Pharmaceutical Biology, University of Bonn, Nussallee 6, D-53115 Bonn, Germany, and Institute of Pharmaceutical Biotechnology, Saarland University, Building C2.3, D-66123 Saarbrücken, Germany

Received September 19, 2008

Myxobacteria are gliding bacteria of the δ-subdivision of the Proteobacteria and known for their unique biosynthetic capabilities. Two examples of a new class of metabolites, myxotyrosides A (**1**) and B (**2**), were isolated from a *Myxococcus* sp. The myxotyrosides have a tyrosine-derived core structure glycosylated with rhamnose and acylated with unusual fatty acids such as (*Z*)-15-methyl-2-hexadecenoic and (*Z*)-2-hexadecenoic acid. The fatty acid profile of the investigated *Myxococcus* sp. (strain 131) is that of a typical myxobacterium with a high similarity to those described for *M. fulvus* and *M. xanthus,* with significant concentrations of neither 15-methyl-2-hexadecenoic acid nor 2-hexadecenoic acid being detected.

Myxotyrosides A (**1**) and B (**2**) are new metabolites isolated from a *Myxococcus* strain, which is a gliding bacterium (myxobacterium) of the δ-subdivision of the Proteobacteria. These microorganisms are frequently encountered in soil, with *Myxococcus xanthus*, whose genome has been sequenced,[1] being the best studied member of this order. *Myxococcus* spp. are unique concerning the diversity of their secondary metabolites;[2,3] for example, they produce nonribosomal peptides such as myxovalargin,[4] acyldepsipeptides such as the myxochromides,[5] and various compounds of mixed PKS/NRPS biosynthetic origin such as myxalamide,[6] myxopyronin,[7] myxothiazol,[8,9] myxovirescin,[10,11] and rhizopodin.[12]

The presence of myxotyrosides (**1**, **2**) in the extracts of *Myxococcus* strain 131 was evident from two distinct peaks in the LC-MS spectra with positive-ESI quasi molecular ion signals at *m*/*z* 532 $[M + H]^+$ and *m*/*z* 518 $[M + H]^+$, respectively. These masses did not correspond to any known myxobacterial metabolite. Myxotyrosides A (**1**) and B (**2**) are a novel class of myxobacteria-derived secondary metabolites, composed of a tyrosine-related core structure, which is glycosylated with rhamnose at the phenolic hydroxy group and acylated with unusual fatty acids, i.e., (*Z*)-15-methyl-2-hexadecenoic and (*Z*)-2-hexadecenoic acid, at the nitrogen functionality. Even though the structural architecture of **1** and **2** is not very complex, the only related molecules are rhamnolipids and similarly acylated amino acids.[13,14]

Glycosylated molecules are rarely found in myxobacterial secondary metabolism, even though exceptions such as chivosazol[15] and the sorangiosids[16] do exist. In these cases the attached sugars are quinovose (6-deoxyglucose) and glucose, respectively. In the myxotyrosides (**1**, **2**) rhamnose is incorporated, a sugar which is widely distributed in nature[17] and is part of the lipopolysaccharides of many prokaryotes including those of myxobacteria.[18]

For myxobacteria the occurrence of iso-branched fatty acids as in **1** is rather typical, and analyses of the fatty acid profiles of *Stigmatella*[19] and *Myxococcus*[20] species showed iso-15:0 (13-methyltetradecanoic acid) to be the most abundant fatty acid. The fatty acid profile of the *Myxococcus* sp. (strain 131) is similar to that of other *Myxococcus* species; however no myxotyrosides with these fatty acids have been found during the current study. This suggests that the biosynthesis of the myxotyrosides does not rely on the fatty acid pool of the producer strain, but involves the *de novo* formation of the myxotyroside-specific fatty acids.

* Corresponding author. Phone: +49 228 733747. Fax: +49 228 733250. E-mail: g.koenig@uni-bonn.de. Internet: http://www.pharma.uni-bonn.de/biologie/index.html.

[†] University of Bonn.

[‡] University of Saarbrücken.

Results and Discussion

Myxococcus sp. (strain 131) was isolated from a soil sample of Sardinia and identified as *Myxococcus* sp. based on morphological characteristics as well as 16S rDNA analysis. After cultivation in liquid medium in the presence of an adsorber resin (Amberlite XAD-16), cell mass and resin were separated from the culture broth by centrifugation and extracted with acetone. Liquid−liquid partitioning and several chromatographic steps (VLC and HPLC, see Experimental Section) yielded myxotyrosides A (**1**) and B (**2**).

The molecular formula $C_{31}H_{49}NO_6$ of myxotyroside A (**1**) was established by LC-ESIMS, NMR spectroscopy, and HREIMS (measured 531.3567, calculated 531.3567). After assignment of all protons and their corresponding carbon atoms by HSQC spectroscopy, spin systems were identified using COSY data, and the resulting partial structures connected making use of key correlations in the HMBC spectrum.

A glycosidic moiety in **1** was evident from ^{13}C NMR chemical shifts of methine carbons C-2′ to C-5′ resonating between δ_C 70.7 and 73.8, which is typical of carbons attached to oxygen. The ^{13}C NMR resonance of C-1′ was shifted further downfield to δ_C 99.8, as expected for an acetal carbon, whereas C-6′, resonating at δ_C 18.0, showed a highfield shift and indicated the presence of a deoxy sugar. The corresponding protons H-1′ (δ_H 5.48, J = 1.8 Hz), H-2′ (δ_H 4.04, J = 1.8 Hz, 3.3 Hz), H-3′ (δ_H 3.88, J = 3.3 Hz, 9.5 Hz), H-4′ (δ_H 3.50, J = 9.5 Hz), H-5′ (δ_H 3.67, J = 6.2 Hz, 9.5 Hz), and H_3-6′ (δ_H 1.26, J = 6.2 Hz) were part of a distinct ^{1}H−^{1}H spin system. An HMBC correlation from H-1′ to C-5′ (δ_C 70.7) proved the site of the acetal function and established the presence of a rhamnose moiety. The chemical shifts of the protons, and more importantly the ^{1}H−^{1}H coupling constants, correspond exactly to those described for α-rhamnose[21] (Figure 4), i.e., ^{1}H−^{1}H coupling constant between H-1′ (δ_H 5.46, J = 1.8 Hz) and H-2′ (δ_H 4.04, J = 1.8 Hz). NOESY correlations confirm the presence of α-rhamnose (Figure 5). All other NMR data are consistent with α-rhamnopyranose, which is the most frequent form of rhamnose in nature.[22] Due to the scarcity of material and in order to be able to perform several bioassays, determination of the absolute configuration was not attempted. It is, however, most likely that the L-form is present, since this enantiomer of rhamnose is most widespread in nature.[17]

An HMBC correlation from H-1′ to C-6 (δ_C 156.6) showed that C-1′ is the link to the tyrosine-derived moiety of **1**. All carbon atoms of the latter showed ^{13}C NMR shifts in the range of olefinic and aromatic carbons (δ_C 112.7 to 131.1), with C-6 being attached to oxygen, as evident from a downfield shift to δ_C 156.6. The aromatic protons H-4/8 (δ_H 7.34, J = 8.8 Hz) and H-5/7 (δ_H 7.11, J = 8.8 Hz) gave rise to a doublet each and evidenced a

10.1021/np8005875 CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 12/29/2008

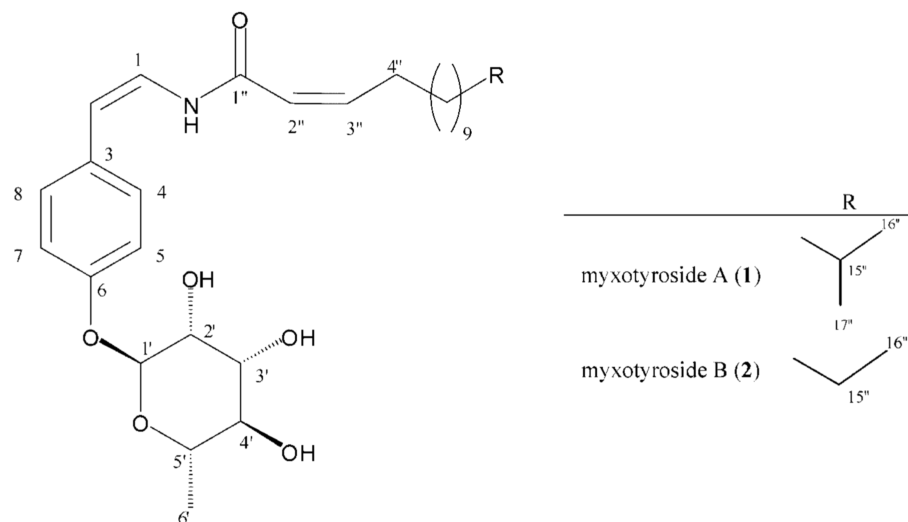


Figure 1. Structures of myxotyrosides A (**1**) and B (**2**).

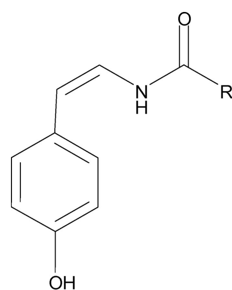


Figure 2. Tyrosine-derived long-chain acyl phenols (R includes unbranched saturated and monounsaturated fatty acids ranging from C_{10} to C_{18}).

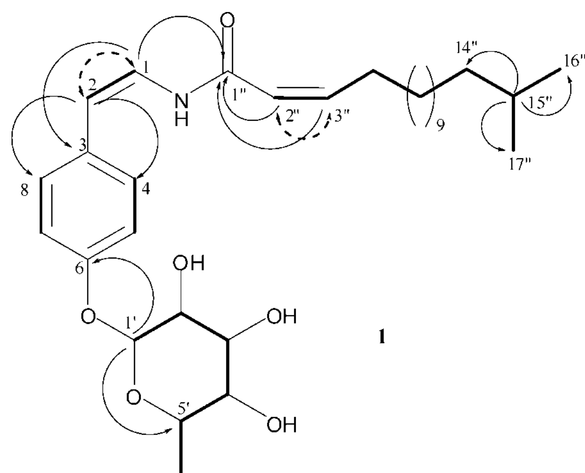


Figure 3. Selected HMBC (solid) and NOESY correlations (dashed). $^1H-^1H$ COSY fragments are highlighted in bold.

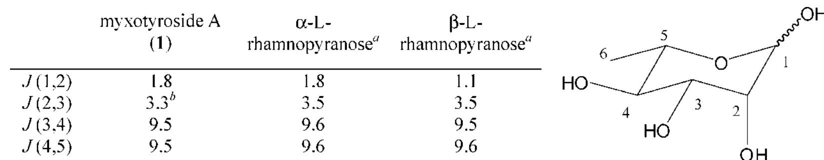
1,4-disubstituted phenyl ring. H-1 (δ_H 6.83, $J = 9.5$ Hz) and H-2 (δ_H 5.77, $J = 9.5$ Hz) formed a further $^1H-^1H$ spin system, and the resulting partial structure is connected to the phenyl residue as proven by HMBC correlations from H-2 to C-4/8 (δ_C 130.7). The $^1H-^1H$ coupling constant between H-1 and H-2 was found to be 9.5 Hz, indicating the double bond $\Delta^{1,2}$ to be *Z*-configured, which was also supported by a NOESY correlation between H-1 and H-2. H-1 showed HMBC correlations to the carbonyl carbon C-1″ (δ_C 166.9), whose ^{13}C NMR shift is characteristic for an amide-bonded carbon. Thus, the methine carbon C-1 is directly attached to nitrogen, completing the tyramine analogous part of **1**.

An iso-branched fatty acid is linked to the tyrosine-derived partial structure via the amide bond. The fatty acid is monounsaturated and the protons of the double bond, i.e., H-2″ (δ_H 6.02) and H-3″ (δ_H 6.18), both showed HMBC correlations to C-1″ and to unresolved ^{13}C NMR resonances of carbons of the methylene chain. Therefore, the double bond is located adjacent to the amide carbonyl group. NOESY correlations between H-2″ and H-3″ and the $^1H-^1H$ coupling constant of $J = 11.7$ Hz showed the double bond $\Delta^{2'',3''}$ to be *Z*-configured. Since the NMR signals of the methylene groups are overlapping, the chain length was deduced from the molecular mass and the corresponding molecular formula. The terminal methyl groups H_3-16″/ H_3-17″ (δ_H 0.91, $J = 6.6$ Hz) couple with H-15″ (δ_H 1.54, $J =$ n.d.), which also shows HMBC correlations to C-16″/17″, C-14″ and to the carbon resonances of the methylene chain. Thus, the fatty acid was identified as (*Z*)-15-methyl-2-hexadecenoic acid.

Myxotyroside B (**2**) is structurally very similar to myxotyroside A, but slightly more polar, and the only difference between the two molecules is the attached fatty acid. LC-MS and HREIMS (measured 517.3412, calculated 517.3403) experiments show a mass difference of 14 between the molecules and indicated a molecular formula of $C_{30}H_{47}NO_6$ for **2**. The mass difference is equivalent to the loss of a CH_2 group in the fatty acid moiety, implying that the fatty acid in compound **2** could either be shorter than the one in compound **1** or have no iso-branching. From the NMR spectra only two methyl groups, i.e., C-6′ of the rhamnose and one belonging to the fatty acid, could be deduced. Additionally, C-15″ is a methylene and not a methine carbon, so that the fatty acid had to be without branching. Therefore, in **2** (*Z*)-2-hexadecenoic acid is incorporated in the molecule.

Myxotyroside A (**1**) was assayed toward a panel of 36 cancer cell lines and shown to be slightly active toward 10 of them at a concentration of 19 μM (mean IC_{50} value 11 μM); however no cytotoxic activity was found at a concentration of 2 μM. Compound **1** showed no antimicrobial activity in a disk diffusion assay against six test organisms (*Bacillus megaterium, Escherichia coli, Eurotium rubrum, Microbotryum violaceum, Mycotypha microspora, Chlorella fusca*) at a concentration of 50 μg/disk. Some antiplasmodial activity ($IC_{50} = 7$ μM) against *Plasmodium falciparum* was observed.

Quorum sensing activities of myxotyrosides A and B were evaluated in biosensor systems using *Escherichia coli* MT102(pSB403), *Pseudomonas putida* F117 pKR-C12, *P. putida* F117 pAS-C8, and *Chromobacterium violaceum*. No activation of acyl-homoserinlactone (AHL) sensing was found; in contrast inhibition could be observed, albeit extremely weak (data not shown).



	myxotyroside A (1)	α-L-rhamnopyranose[a]	β-L-rhamnopyranose[a]
J(1,2)	1.8	1.8	1.1
J(2,3)	3.3[b]	3.5	3.5
J(3,4)	9.5	9.6	9.5
J(4,5)	9.5	9.6	9.6

Figure 4. Vicinal ^{1}H−^{1}H NMR coupling constants (in Hz) of myxotyroside A (**1**) (MeOD) and rhamnose (D_2O).

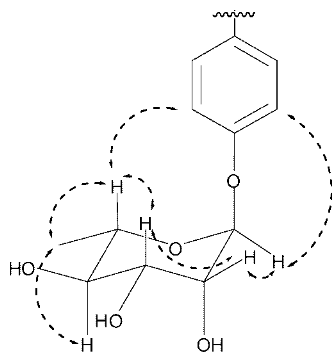


Figure 5. Selected ^{1}H−^{1}H NOESY correlations supporting the α-configuration of the rhamnose unit.

Moreover, we tested the influence of **1** and **2** on the fruiting body formation of *M. xanthus* DK1622, but no difference from a MeOH-treated control could be observed (data not shown).

The fatty acid profile (Table 2) of strain 131 is that of a typical myxobacterium with a high similarity to that described for *Myxococcus fulvus*.[20] Compared to the fatty acid profile of the model strain *Myxococcus xanthus* DK1622, the most prominent differences are the considerably lower concentration of iso-15:0 and the slightly elevated concentration of 16:1ω5c, 16:0, iso-17:0, and iso-17:0 2-OH. Neither (*Z*)-15-methyl-2-hexadecenoic acid nor (*Z*)-2-hexadecenoic acid were present in significant concentrations.

The myxotyrosides are new metabolites composed of a modified tyrosine and fatty acid moiety, as well as the deoxysugar rhamnose. Such an assembly of building blocks is novel, making the myxotyrosines the first members of a group of bacterial metabolites. Acylated phenols have been isolated once before using a metagenomic approach;[13,14,23] in this case, however, the producer organisms are not known, and structurally the latter compounds lack the glycosidic part and the nature of the incorporated fatty acids is different. Rhamnosides[17] and even rhamnolipids[24] are widespread in nature, and especially the latter are frequently encountered in microorganisms; yet, the combination with an amino acid-related moiety is unique.

The fatty acid profile of *Myxococcus* strain 131 showed that (*Z*)-15-methyl-2-hexadecenoic (iso-17:1) and (*Z*)-2-hexadecenoic acid (16:1), the two fatty acid moieties in **1** and **2**, are not detectable from whole cell fatty acid compositions in this microorganism. Thus, the biosynthesis of **1** and **2** supposedly involves the specific formation of the acyl part of **1** and **2**, concomitantly with the production of the tyramine-related moiety. The biosynthetic gene cluster might be envisaged as a hybrid PKS/NRPS. Concerning the PKS/fatty acid-derived part of structure **1**, three different pathways leading to 15-methyl-2-hexadecenoic acid seem plausible: first the dehydration of 2-hydroxy-15-methylhexacadecanoic acid (iso-17:0 2-OH, with an exceptionally high concentration in strain 131); second using 13-methyltetradecanoic acid (iso-15:0), which usually is the most abundant fatty acid in myxobacteria,[19,20] as starter followed by elongation, ketoreduction, and dehydration steps. The result of the fatty acid analysis, revealing a high content of 2-hydroxy-15-methylhexacadecanoic acid (Table 2), makes the former pathway more probable. As a third possibility, both fatty acids in **1** and **2** might be derived from a desaturase-catalyzed oxidation of the corresponding saturated fatty acids. Although no such desaturase has been described to date, 13 different desaturase-encoding genes have been identified in *M. xanthus* DK1622 (Bode, unpublished), of which one might show the required regioselectivity.

It can be assumed that the myxotyrosides have a beneficial function for the producing organism. Antibiotic, toxic, and lytic secondary metabolites of heterotrophic bacteria may be used to kill and degrade prey organisms or competitors and thus have a benefit for the producer. Other secondary metabolites may serve as siderophores, repellents, or signaling molecules.[25] The results of the antimicrobial and cytotoxicity assays, however, showed that the myxotyrosides are not active toward bacteria and fungi, at least toward the organisms applied in the assays. Rhamnolipids structurally related to myxotyrosides are reputed to be part of an antiprotozoal defense.[25] The antiplasmodial activity of myxotyrosides also points in that direction and should be further investigated, since predation by protozoans is a substantial threat to bacterial communities.[26] This might be of special importance for myxobacterial fruiting bodies and spores, which can survive for a long time in the soil, but are unable to move away from such predators, as they are nonmotile.

For the biosynthesis of the myxotyroside-related acylated phenols, which were derived from expression of a metagenomic library in *E. coli*,[13,14,23] the involvement of an *N*-acyl amino acid transferase (FeeM) with structural similarity to acyl homoserine lactone synthases was demonstrated by X-ray analysis.[27] For these acylated phenols, it has therefore been discussed that their function may be some kind of signaling.[23] Acyl homoserine lactones (AHL), like myxotyrosides, are *N*-acylated compounds, and they are well known as quorum sensing signaling molecules in a range of proteobacteria.[28,29] This led to the suggestion that the myxotyrosides might play a role in bacterial communication systems. Evaluation of **1** and **2** in bacterial biosensor systems for quorum sensing activities showed them, however, to be inactive. This is not surprising since these biosensors are specific for acyl homoserine lactones, and our results merely demonstrate that myxotyroside A is not involved in AHL-related signaling. Communication systems of myxobacteria have been proven to be exceptional,[1,30,31] and the possible involvement of myxotyrosides as signaling molecules needs to be addressed in future studies.

Structurally unusual fatty acids, similar to those found in the myxotyrosides, have been discussed as signaling molecules,[32,33] and iso-branched fatty acids have been proven to have a major influence on aggregation and sporulation and thus on fruiting body formation.[32] Therefore, we analyzed the influence of **1** and **2** on fruiting body formation in *M. xanthus* DK1622. However, as we did not observe any difference (growth inhibition or inhibition or acceleration of fruiting body formation) from the control experiment, one might speculate either that there is no such role of **1** and **2** or that their function is only very specific for the producing strain. According to the latter hypothesis, no secondary metabolite gene cluster has been identified in DK1622 that would fit the proposed biosynthesis of **1** and **2** (Bode, unpublished and ref 1).

In summary, the myxotyrosides are a novel structural type of myxobacterial metabolites, whose function for the producer organism will be part of future studies.

Experimental Section

General Experimental Procedures. Optical rotation measurements were conducted on a Jasco model DIP-140 polarimeter (1 dm, 1 cm^3 cell) operating at $\lambda = 589$ nm, corresponding to the sodium D line at

Table 1. NMR Spectroscopic Data (300 MHz, CD_3OD) for Myxotyroside A (**1**)

position	δ_C, mult	δ_H (*J* in Hz)	HMBC	COSY	NOESY
1	121.8, CH	6.83, d (9.5)	2, 3, 1″	2	2
2	112.7, CH	5.77, d (9.5)	1, 4 + 8	1	1, 4 + 8
3	131.1, qC				
4 + 8	130.7, CH	7.34, d (8.8)	2, 6, 4 + 8, 5 + 7	2, 5 + 7	2, 5 + 7
5 + 7	117.7, CH	7.11, d (8.8)	6, 4 + 8, 5 + 7	4 + 8	4 + 8, 1′, 5′
6	156.6, qC				
1′	99.8, CH	5.48, d (1.8)	6, 5′	2′	5 + 7, 2′
2′	72.1, CH	4.04, dd (1.8, 3.3)	2′, 3′, 4′, 5′, 6′	1′, 3′	1′, 3′
3′	72.2, CH	3.88, dd (3.3, 9.5)	2′, 4′, 5′	1′, 2′, 4′	2′, 5′
4′	73.8, CH	3.50, t (9.5)	2′, 4′, 5′	3′, 5′	6′
5′	70.7, CH	3.67, dd (6.2, 9.5)	1′, 4′, 6′	4′, 6′	5 + 7, 3′, 6′
6′	18.0, CH_3	1.26, d (6.2)	4′, 5′	5′	4′, 5′
1″	166.9, qC				
2″	122.4, CH	6.02, dt (11.7, 1.1)	1″, 3″, 4″	3″	3″
3″	149.5, CH	6.18, dt (11.7, 7.3)	1″, 2″, 4″	2″	2″, 4″, 5″−13″
4″	30.5, CH_2	2.74, dt (7.3, 1.1)	2″, 3″, 5″−13″		5″−13″
5″−13″	28.6−31.5, CH_2	1.3−1.5		4″, 5″−13″	4″, 5″−13″
14″	40.3, CH_2	1.20, m	5″−13″, 15″	5″−13″	5″−13″
15″	29.2, CH	1.54, m	5″−13″, 14″, 16″ + 17″	14″, 16″ + 17″	5″−13″
16″ + 17″	23.1, CH_3	0.91, d (6.6)	5″−13″, 14″, 16″ + 17″	15″, 16″ + 17″	5″−13″

Table 2. Fatty Acid Analysis of *Myxococcus* sp. (strain 131) and *Myxococcus xanthus* (DK1622)

	content (%) in strain	
fatty acid	131	DK1622
12:0		
iso-13:0	0.46	0.30
iso-14:0		
14:1 isomer 1		1.22
14:1 isomer 2		
14:0	0.88	6.43
iso-15:1ω9c		
iso-15:1 isomer 2		
iso-15:0	25.14	60.71
15:0		1.25
15:1 isomer 1		1.45
15:1 isomer 2		1.18
iso-16:0	0.41	
16:2ω5c,11c		3.55
16:1ω11c		
16:1ω5c	29.57	9.13
16:0	8.12	1.45
iso-17:2ω5c,11c		1.75
iso-17:1ω11c		
iso-17:1ω5c	2.00	
iso-17:0	18.06	2.85
14:0 3-OH	0.11	
iso-15:0 3-OH	1.11	1.29
16:0 2-OH	0.32	0.13
16:0 3-OH		
iso-17:0 2-OH	11.07	4.59
iso-17:0 3-OH		1.45
iso-15:0 (dimethylacetal)	0.82	0.98
iso-15:0 (*O*-alkyl ether)	1.94	0.29

room temperature. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer with CD_3OD as the solvent and internal standard. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 for CD_3OD. EIMS were recorded using a Finnigan MAT 95 spectrometer. LC-ESIMS was performed using an Agilent 1100 system with an API 2000 Triple Quadrupole LC/MS/MS (Applied Biosystems/MDS Sciex) and a DAD. Analytical TLC: TLC aluminum sheets, silica gel Si 60 F254 (Merck), solvent system DCM/MeOH (9:1). Detection: quenching of fluorescence at 254 nm and fluorescence at 366 nm and detection with vanillin/H_2SO_4 reagent and heating to 110 °C. HPLC was carried out using either an HP system equipped with a 1050 pump, a 1050 autosampler, and a 1050 multiple wavelength detector or a system consisting of a Waters associated chromatography pump, a Rheodyne 7725i injection system, a Knauer differential refractometer, and a Linseis L200E recorder.

Isolation and Taxonomy of the Bacterial Strain. The myxobacterial strain (no. 131) was isolated from a soil sample that was collected on the island of Sardinia. Small amounts of the sample were applied onto WCX-*E.coli*-agar plates, on which the strain was isolated. It was transferred on VY/2 agar plates until an axenic culture was obtained. The morphology of the swarm (film-like swarm that neither corroded the agar nor showed extreme radial veins) and the microscopical appearance of the vegetative cells (slender rods, cigar shaped) led to the assumption that strain 131 belongs to the genus *Myxococcus*. The taxonomy could be confirmed by 16S rDNA analysis. Stock cultures of the strain are kept at −80 °C.

Isolation Procedure. Cultivation was performed in 10 5-L Erlenmeyer flasks, each containing 1.5 L of a peptone medium (MD1 medium supplemented with 0.2% starch and 0.2% glucose × H_2O) with 2% Amberlite XAD-16 (Fluka, Germany). MD1 medium consists of Casiton 3 g/L, $CaCl_2 \times 2H_2O$ 0.7 g/L, $MgSO_4 \times 7H_2O$ 2 g/L. The flasks were inoculated with a preculture (same medium, 100 mL) and shaken on a rotary shaker (140 rpm) at 30 °C for seven days. At the end of the cultivation, the bacterial cells and adsorber resin were separated from the culture broth by centrifugation and extracted with acetone. After removal of the solvent, the residue (5.75 g) was suspended in 60% aqueous MeOH and extracted six times with CH_2Cl_2 (50 mL). The CH_2Cl_2 layers were combined and dried (1.62 g). Separation of this extract by vacuum liquid column chromatography (VLC) over reversed-phase silica gel (approximately 20 g) using MeOH/H_2O mixtures from 50/50 to 100% MeOH and then 100% CH_2Cl_2 as eluents gave nine fractions. TLC and ^{1}H NMR analyses showed the compounds to be present in fraction 6 (100% MeOH). This fraction (178 mg) was subjected to two subsequent HPLC separations. The first preseparation was carried out on a normal-phase column (250 × 8 mm, 5 μM Eurospher-100 Si, Knauer) with petroleum ether/acetone (70/30) as solvent system and a flow rate of 2 mL/min. The retention time of the impure mixture of **1** and **2** (29 mg) was 4.5 min. This mixture was then separated on a reversed-phase column (C-8, 250 × 8 mm, 5 μm Eurospher-100 C_8, Knauer) with MeOH/H_2O (80/20) as solvent system with a flow rate of 2.5 mL/min. Thus, 7.1 mg of pure compound **1** (retention time 17.7 min) and 6.6 mg of compound **2** (retention time 15.7 min) could be obtained.

Myxotyroside A (1): colorless solid; $[\alpha]^{20}_D$ −71.4 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 nm (4.22), 297 nm (4.26); IR ν_{max} 3335, 2922, 2851, 2361, 1648, 1476, 1233, 1124, 1061, 1020, 982, 839, 668, 631 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* (%) 531 (10), 385 (75), 135 (100); HREIMS *m/z* 531.3567 (calcd for $C_{31}H_{49}NO_6$ 531.3567).

Myxotyroside B (2): colorless solid; $[\alpha]^{20}_D$ −79.8 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 nm (4.22), 297 nm (4.28); IR ν_{max} 3334, 2921, 2852, 2357, 1649, 1476, 1233, 1124, 1061, 1020, 982, 839, 667 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Supporting Information; EIMS *m/z* (%) 517 (10), 371 (75), 135 (100); HREIMS *m/z* 517.3412 (calcd for $C_{30}H_{47}NO_6$ 517.3403).

Fatty Acid Profile. The fatty acid analysis was performed by GC and GC-MS after saponification, methylation, and extraction.[32]

Bioactivity. Cytotoxicity,[34] antimicrobial,[35] quorum sensing,[36] and antiplasmodial[37] assays were carried out according to literature procedures. For analysis of the influence of **1** and **2** on fruiting body formation, large-scale cultivation of fruiting bodies was performed as described previously,[38] and 0.5 mg of compounds **1** and **2** was applied on filter disks as in agar diffusion assays and compared with a MeOH-treated filter disk. Fruiting body formation was observed microscopically for three days, and photos were taken after 6, 24, 48, and 72 h. Differences that have been observed with other compounds were growth inhibition and inhibition or acceleration of fruiting body formation.

Acknowledgment. We thank E. Neu for technical assistance and for performing the disk diffusion assay, Ms. C. Sondag, Department of Chemistry, University of Bonn, for the EIMS measurements, and S. Andes for help with the fruiting body formation assay. Further thanks go to Ö. Erol-Hollmann for help with the taxonomy of the strain. For the bioactivity evaluation we are grateful to Prof. Dr. R. Brun, Swiss Tropical Institute, Basel, Prof. Dr. Fiebig and Dr. Maier, Oncotest GmbH, Freiburg, Prof. Dr. L. Eberl and PD Dr. K. Riedel, University of Zurich, and their respective research groups.

Supporting Information Available: NMR spectra of **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Goldman, B. S.; Nierman, W. C.; Kaiser, D.; Slater, S. C.; Durkin, A. S.; Eisen, J. A.; Ronning, C. M.; Barbazuk, W. B.; Blanchard, M.; Field, C.; Halling, C.; Hinkle, C.; Iartchuk, O.; Kim, H. S.; Mackenzie, C.; Madupu, R. R.; Miller, N.; Shvartsbeyn, A.; Sullivan, S. A.; Vaudin, M.; Wiegand, R.; Kaplan, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15200–15205.

(2) Gerth, K.; Pradella, S.; Perlova, O.; Beyer, S.; Müller, R. *J. Biotechnol.* **2003**, *106*, 233–253.

(3) Reichenbach, H.; Höfle, G. *Biotech. Adv.* **1993**, *11*, 219–277.

(4) Irschik, H.; Gerth, K.; Kemmer, T.; Steinmetz, H.; Reichenbach, H. *J. Antibiot.* **1983**, 6–12.

(5) Trowitzsch-Kienast, W.; Gerth, K.; Wray, V.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1993**, *12*, 1233–1237.

(6) Jansen, R.; Reifenstahl, G.; Gerth, K.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1983**, *7*, 1081–1095.

(7) Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1983**, *10*, 1656–1667.

(8) Gerth, K.; Irschik, H.; Reichenbach, H.; Trowitzsch, W. *J. Antibiot.* **1980**, 1474–1479.

(9) Trowitzsch, W.; Reifenstahl, G.; Wray, V.; Gerth, K. *J. Antibiot.* **1980**, 1480–1490.

(10) Gerth, K.; Irschik, H.; Reichenbach, H.; Trowitzsch, W. *J. Antibiot.* **1982**, *45*, 1454–1459.

(11) Trowitzsch, W.; Wray, V.; Gerth, K.; Höfle, G. *J. Chem Soc., Chem. Commun.* **1982**, 1340–1342.

(12) Sasse, F.; Steinmetz, H.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **1993**, *46*, 741–748.

(13) Brady, S. F.; Chao, C. J.; Clardy, J. *J. Am. Chem. Soc.* **2002**, *124*, 9968–9969.

(14) Brady, S. F.; Clardy, J. *J. Am. Chem. Soc.* **2000**, *122*, 12903–12904.

(15) Irschik, H.; Jansen, R.; Gerth, K.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **1995**, *48*, 962–966.

(16) Irschik, H.; Jansen, R.; Gerth, K.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **1986**, *40*, 7–13.

(17) Monti, D.; Pisvejcová, A.; Kren, V.; Lama, M.; Riva, S. *Biotechnol. Bioeng.* **2004**, *87*, 763–771.

(18) Rosenfelder, G.; Lüdertz, O.; Wetsphal, O. *Eur. J. Biochem.* **1974**, *44*, 411–420.

(19) Schröder, J.; Reichenbach, H. *Arch. Mikrobiol.* **1970**, *71*, 384–390.

(20) Ware, J. C.; Dworkin, M. *J. Bacteriol.* **1973**, *115*, 253–261.

(21) Altona, C.; Haasnoot, C. A. G. *Org. Magn. Reson.* **1980**, *13*, 417–429.

(22) Dewick, P. M. *Medicinal Natural Products*, 2nd ed.; Wiley-VCH: New York, 2001; p 469.

(23) Brady, S. F.; Clardy, J. *Org. Lett.* **2005**, *7*, 3613–3616.

(24) Nitschke, M.; Costa, S. G. V. A. O.; Contiero, J. *Biotechnol. Prog.* **2005**, *21*, 1593–1600.

(25) West, S. A.; Diggle, S. P.; Buckling, A.; Gardner, A.; Griffin, A. S. *Annu. Rev. Ecol. Evol. Syst.* **2007**, *38*, 53–77.

(26) Matz, C.; Kjelleberg, S. *Trends Microbiol.* **2005**, *13*, 302–307.

(27) Van Wagoner, R. M.; Clardy, J. *Structure* **2006**, *14*, 1425–1435.

(28) Visick, K. L.; Fuqua, C. *J. Bacteriol.* **2005**, *187*, 5507–5519.

(29) Fuqua, C.; Greenberg, E. P. *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 685–95.

(30) Zusman, D. R.; Scott, A. E.; Yang, Z.; Kirby, J. R. *Nat. Rev. Microbiol.* **2007**, *5*, 862–72.

(31) Kaiser, D. *Annu. Rev. Microbiol.* **2004**, *58*, 75–98.

(32) Bode, H. B.; Ring, M. W.; Kaiser, D.; David, A. C.; Kroppenstedt, R. M.; Schwär, G. *J. Bacteriol.* **2006**, *188*, 5632–5634.

(33) Curtis, P. D.; Shimkets, L. J. In *Myxobacteria: Multicellularity and Differentiation*; Whithworth, D. E., Ed.; ASM Press: Washington, DC, 2008; p 251.

(34) Fiebig, H. H.; Berger, D. P.; Dengler, W. A.; Wallbrecher, E.; Winterhalter, B. R. *Contributions in Oncology*; Karger Verlag: Basel, 1992; Vol. 42, pp 321−351.

(35) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007–1015.

(36) Krick, A.; Kehraus, S.; Eberl, L.; Riedel, K.; Anke, H.; Kaesler, I.; Graeber, I.; Szewzyk, U.; König, G. M. *Appl. Environ. Miocrobiol.* **2007**, *73*, 3587–3594.

(37) Kayser, O.; Kiderlen, A. F.; Brun, R. *Planta Med.* **2001**, *67*, 718–721.

(38) Ring, M. W.; Schwär, G.; Thiel, V.; Dickschat, J. S.; Kroppenstedt, R. M.; Schulz, S.; Bode, H. B. *J. Biol. Chem.* **2006**, *281*, 36691–36700.

NP8005875