Myxochromide B_3, a New Member of the Myxochromide Family of Secondary Metabolites

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Myxochromides are cyclic depsipeptides with an unsaturated polyketide side chain, which have been reported from different myxobacterial species, e.g., *Myxococcus xanthus* and *Stigmatella aurantiaca*. To date, myxochromides are subdivided into the groups A and S, according to their peptidic core structure. The peptide moiety of the new myxochromide B_3 (**1**), which was isolated from a myxobacterial strain of the genus *Myxococcus*, differs from that of myxochromides A and S. Compound **1** thus is the first representative of a new group of myxochromides. For myxochromide A_3 (**2**) the complete and assigned spectroscopic data are described. For the structure elucidation one- and two-dimensional NMR spectroscopy as well as mass spectrometry have been applied. Configurational analysis has been accomplished by chiral GC-MS and HPLC.

Myxobacteria are gliding bacteria that belong to the δ-subdivision of the Proteobacteria. They 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 outstanding 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 a mixed PKS/NRPS biosynthetic origin such as myxalamide,[6] myxopyronin,[7] myxothiazol,[8,9] myxovirescin,[10,11] and rhizopodin.[12]

Myxochromides are cyclic depsipeptides with acyl polyene side chains and are divided into two subgroups, called myxochromides A and S.[13,14] Compounds of each subgroup share the peptidic core structure, but differ with respect to the polyene moiety. The peptidic part of members of the A series consists of six amino acids, namely, two alanine residues, proline, leucine, *N*-Me-threonine, and glutamine. The cyclic core of myxochromides S does not include a proline residue and thus consists of only five amino acids, i.e., leucine, two alanines, *N*-Me-threonine, and glutamine (Figure 1). The amino acid sequence of the A and S series differs regarding the position of the alanine and leucine residues, which is interchanged. Here we describe the first member of a new subgroup of myxochromides, named myxochromides B.

The polyene side chain of myxochromides varies in length and in the number of double bonds. This structural feature of the myxochromides is expressed in the subscript number of the names; for example, myxochromide S_2 and A_2 have the same polyene carboxylic acid attached (Figure 1).

In the literature, the naming and in part also the structures given for certain myxochromides are confusing. Trowitzsch-Kienast et al.[5] had first isolated a number of myxochromides and named them myxochromides A to E. This report, however, merely described the detailed structure of one of them, i.e., myxochromide A_2, whereas the structures of myxochromides B to E were assumed to be identical concerning the peptidic part, but different with regard to the polyene moiety. In an attempt to find a coherent nomenclature and to clarify structural issues for the myxochromides, Wenzel et al.[14] renamed myxochromides A−E, which all share the same cyclic core, to myxochromides A_{2-4}. Of the myxochromides of the S series five members were described, i.e., myxochromides S_{1-3}, from *Stigmatella aurantiaca*[13] and myxochromides S_{4-5} from *Myxococcus xanthus*. The latter, however, appear in Antibase with only a poster presentation (Wenzel et al.) as the reference.

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In our LC-MS screening program, the extracts of *Myxococcus* strains 163 and 171 were found to show positive-ESI quasimolecular ion signals at *m/z* 846 $[M + H]^+$ for strain 163 and *m/z* 959 $[M + H]^+$ for strain 171. Dereplication analysis of the MS data did not lead to positive hits (Antibase, Scifinder), suggesting the presence of two, to date unknown, natural products. Compound **1** from *Myxococcus* strain 171 was found to have a new depsipeptide core structure and therefore represents the first member of a new myxochromide group. Compared to members of the A series, it contains an additional leucine residue, but the same polyene chain as myxochromide A_3 (2,4,6,8,10,12,14,16-octadecaoctaenoic acid); thus we named it myxochromide B_3 (**1**) (Figure 1). Myxochromide A_3 (**2**), which we obtained from strain 163, is not a new natural product; however it is not listed in Scifinder, even though it is described by Wenzel et al.[14] Here we present the complete spectroscopic data for compound **2**.

Results and Discussion

Two myxobacterial strains (nos. 171 and 163) were isolated from a soil sample collected in Brittany, France, and identified as *Myxococcus* sp., on the basis of 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 adsorber 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 myxochromide B_3 (**1**) and myxochromide A_3 (**2**).

Compound **1** has the molecular formula $C_{51}H_{74}N_8O_{10}$ as determined by HR-ESIMS (981.5418, calculated $[M + Na]^+$ 981.5420), which implied 19 degrees of unsaturation. ^1H and ^{13}C NMR spectra showed characteristic resonance signals for multiple olefinic protons (δ_H 5.79−7.39, δ_C 120.0−145.6), indicating a polyene moiety (Table 1). The peptidic structure was evident from resonances for α-CH groups (δ_H 4.15−5.60, δ_C 49.9−63.8), and a sharp singlet for an *N*-CH_3 group (δ_H 3.30) indicated the presence of an *N*-methylated amino acid. After assignment of all protons to their directly bonded carbons with the help of a $^1H-^{13}C$ HSQC spectrum, the amino acids of the peptidic part were identified by $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC data.

$^1H-^1H$ COSY correlations gave evidence of the methylene groups H_2-3 (δ_H 2.38, 1.94), H_2-4 (δ_H 2.12), and H_2-5 (δ_H 3.89, 3.72) and the methine proton H-2 (δ_H 4.15), which coupled to each other. H-2 and H_2-3 showed $^1H-^{13}C$ HMBC correlations to the carbonyl C-1 (δ_C 174.3), which, in conjunction with the corresponding carbon resonances (Table 1), indicated a proline residue. The amino acid leucine, which is present twice, was characterized

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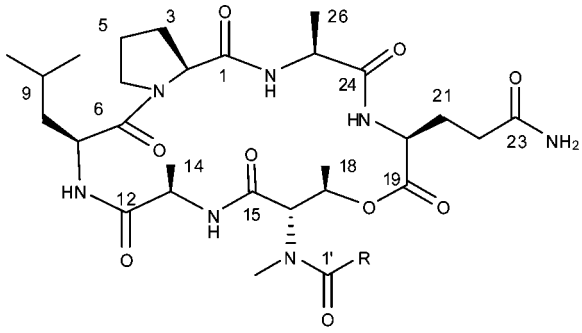

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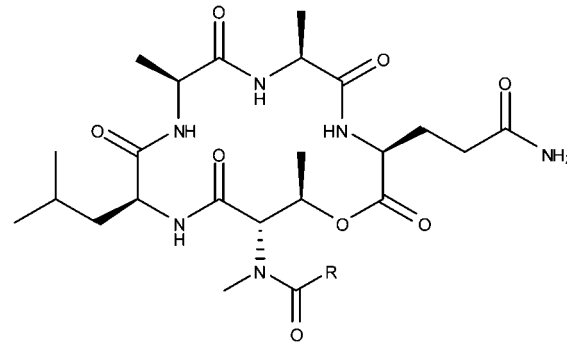

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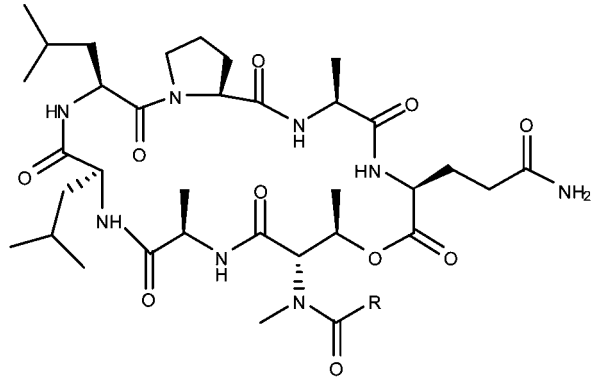

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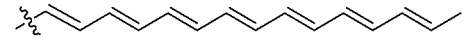
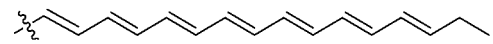
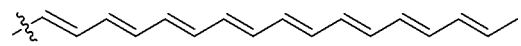
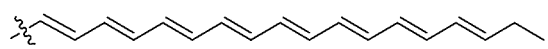
1	
2	
3	
4	

Figure 1. Structures of the myxochromides, including myxochromide B_3 (**1**) and myxochromide A_3 (**2**).

by the $^1H-^1H$ spin systems consisting of the α-CH-groups (CH-7 (δ_C 49.9, δ_H 4.96) and CH-13 (δ_C 55.0, δ_H 4.24)) and the aliphatic protons H_3-11, H_3-10, H-9, and H_2-8 for leucine I and H_3-17, H_3-16, H-15, and H_2-14 for leucine II. Protons of each of the two spin systems showed $^1H-^{13}C$ HMBC correlations to the corresponding carbonyl group, i.e., C-6 (δ_C 174.4) for leucine I and C-12 (δ_C 174.7) for leucine II. Two alanine residues were present in the molecule, as evident from $^1H-^1H$ COSY correlations of the methyl groups CH_3-20 (δ_C 20.0, δ_H 1.32) and CH_3-32 (δ_C 16.8, δ_H 1.46) to the neighboring α-CH groups, CH-19 (δ_C 50.0, δ_H 4.67) and CH-31 (δ_C 50.7, δ_H 4.29), respectively. The methyl protons H_3-20 and H_3-32 as well as the α-protons H-19 and H-31 showed $^1H-^{13}C$ HMBC correlations to the carbonyl group of the amino acid [C-18 (δ_C 174.6) and C-30 (δ_C 175.0)]. The *N*-methyl-threonine part of the molecule was deduced as follows: the carbonyl carbon C-21 (δ_C 168.8) was connected to the α-CH group CH-22 (δ_C 61.4, δ_H 5.60), as could be seen from the $^1H-^{13}C$ HMBC data (Table 1). H-22 coupled with the methine proton H-23 (δ_H 5.88), which also coupled with the methyl group CH_3-24 (δ_H 1.15). The signal of H-23 was shifted downfield, as there was an oxygen attached to the same carbon; the ^{13}C NMR shift of carbon C-23 (δ_C 71.5) was in good accordance with this deduction. The 22-*N*-CH_3 (δ_H 3.30) group clearly belonged to the threonine part of the molecule, as proven by $^1H-^{13}C$ HMBC correlations from the *N*-CH_3 protons to C-22. The nitrogen at C-22 also connected the polyene chain (see below) to the peptidic part of the molecule. The last amino acid comprised two carbonyl groups, C-25 (δ_C 171.0) and C-29 (δ_C 177.4). The $^1H-^1H$ COSY correlations between the two methylene groups CH_2-27 (δ_C 28.8, δ_H 2.07 br) and CH_2-28 (δ_C 32.6, δ_H 2.46, 2.31) showed that they were in adjacent positions. Both had strong $^1H-^{13}C$ HMBC correlations to C-29, and one of them, H_2-27, also to C-25. The same methylene group coupled with the α-CH proton H-26 (δ_H 4.15), which confirmed the presence of a glutamine residue.

The thus established amino acid moieties were connected making use of $^1H-^{13}C$ HMBC and ROESY data. $^1H-^{13}C$ HMBC correlations between H-23 and C-25 proved the neighboring positions of *N*-Me-threonine and glutamine, which are connected via an ester bond. These deductions secured the C-21 to C-29 part of the molecule, including the site of acylation (C-1′). The connection of alanine I to *N*-methyl-threonine and to leucine II was also proven by $^1H-^{13}C$ HMBC data, i.e., by correlations from H-19 to C-21 and from H-13 to C-18, respectively, delineating the C-12 to C-24 part of the molecule. Thus the amino acid sequence from leucine II to glutamine via alanine I and *N*-methyl-threonine was determined. The remaining sequence was based on strong ROESY correlations: several proton resonances of leucine I, i.e., H-7, H-9, and H_3-11, to H_2-5 of proline proved the neighborhood of these two amino acids. At this point of the structure elucidation, alanine II could be located only between the two leucine residues or

Table 1. NMR Spectroscopic Data (500 MHz, CD_3OD) for Myxochromide B_3 (**1**)

	position	δ_C, mult.	δ_H (J in Hz)	COSY	HMBC	ROESY
L-proline	1	174.3, qC				
	2	63.8, CH	4.15, t (8.5)	3a, 3b	1, 3, 4	3a, 3b, 4, 32
	3a	30.8, CH_2	2.38, m	2, 3b, 4	1, 4, 5	2, 3a, 5a, 5b
	3b	30.8, CH_2	1.94, m	2, 3a, 4	1, 2, 4, 5	2, 3a, 5a, 5b
	4	26.2, CH_2	2.12, m	2, 3a, 3b, 4, 5a, 5b	2, 3	2, 5a, 5b
	5a	48.7, CH_2	3.89, m	4, 5b	3, 4	3a, 3b, 4, 7, 9, 11
	5b	48.7, CH_2	3.72, m	4, 5a		3a, 3b, 4, 7, 9, 11
L-leucine I	6	174.4, qC				
	7	49.9, CH	4.96, n.d.[a,d]	8b	4, 6, 8	5a, 5b, 8, 9, 11
	8a	41.2, CH_2	1.66, m	7, 8b, 9, 10, 11	7, 9, 10, 11	7, 10, 11
	8b	41.2, CH_2	1.54, m	7, 8a, 9, 10, 11		
	9	26.0, CH	1.67, m	7, 8a, 8b, 10, 11	8, 10, 11	5a, 5b, 7
	10	23.9, CH_3	0.99, d (6.6)	9	8, 9, 11	8a, 8b, 11
	11	21.4, CH_3	1.02, d (6.6)	8a, 8b, 9	8, 9, 10	5a, 5b, 7, 10
L-leucine II	12	174.7, qC				
	13	55.0, CH	4.24, dd (5.1, 9.2)	14	12, 14, 15, 18	14, 16, 20
	14	41.8, CH_2	1.73, m	13, 15, 17, 16	12, 13, 15, 16, 17	13, 16, 17
	15	26.3, CH	1.79, m	14, 16, 17	13, 14, 16, 17	
	16	21.6, CH_3	0.95, d (6.6)	15	14, 15	13, 14
	17	23.3, CH_3	1.04, d (6.6)	14, 15	14, 15	
alanine I	18	174.6, qC				
	19	50.0, CH	4.67, q (7.0)	20	18, 20, 21	20, 22-*N*CH_3
	20	20.0, CH_3	1.32, d (7.0)	19	18, 19	13, 19
N-Me-threonine	21	168.8, qC				
	22	61.4, CH	5.60, d (3.8)	23	1′, 21	19, 22-*N*CH_3, 23, 24, 2′
	22-*N*CH_3	35.8, CH_3	3.30, s		1′, 22	2′, 23, 2[c], 26[c], 22, 27, 24
	23	71.5, CH	5.88, m	22, 24	24, 25	22, 22-*N*CH_3, 24
	24	16.4, CH_3	1.15, d (6.6)	23	22, 23	22, 22-*N*CH_3, 23
L-glutamine	25	171.0, qC				
	26	55.7, CH	4.15, t (8.5)	27	25, 27, 28	22-*N*CH_3, 28, 32
	27	28.8, CH_2	2.07, m	26, 28	29, 25, 26, 28	19, 22-*N*CH_3, 24, 28
	28a	32.6, CH_2	2.46, m	27, 28b	26, 27, 29	26
	28b	32.6, CH_2	2.31, m	27, 28a	26, 27, 29	26
	29	177.4, qC				
alanine II	30	175.0, qC				
	31	50.7, CH	4.29, q (7.3)	32	1, 30, 32	32
	32	16.8, CH_3	1.46, d (7.3)		30, 31	2[c], 26[c], 31
polyene side chain	1′	171.1, qC				
	2′	120.1, CH	6.74, d (15.8)	3′	1′	5′−16′, 22, 22-*N*CH_3
	3′	145.6, CH	7.39, dd (11.6, 15.8)	2′, 4′	1′, 2′	
	4′	131.6, CH	6.58, m	3′	2′	
	5′−16′	131.3−142.2,CH	6.1−6.8, m[b]	4′	17′, 18′	
	17′	131.1, CH	5.79, m	18′	18′	18′
	18′	18.5, CH_3	1.82, d (6.3)	17′	17′	17′

[a] Signal partly overlapping with the signal of H_2O in the solvent. [b] Signals overlapping. [c] ^1H NMR signals of H-2 and H-26 are superimposed. [d] n.d. = not determined.

between glutamine and proline. As there was a ROESY correlation from the methyl protons H_3-32 of alanine II and the α-CH-protons of proline (δ_H 4.15, CH-2) and/or glutamine (δ_H 4.15, CH-26), alanine II was placed next to glutamine and proline. From the high-resolution mass measurements and the resulting molecular formula $C_{51}H_{74}N_8O_{10}$ the cyclic nature of the peptide was evident. The established amino acid sequence for myxochromide B_3 (**1**) was in accordance with that of the myxochromides of the A series, the only difference being the additional leucine residue.

H-22 as well as the protons of the 22-*N*-methyl group showed $^1H-^{13}C$ HMBC correlations to C-1′, which gave evidence for the polyene chain being attached via *N*-22 of threonine. From the mass spectrometry data and the resulting molecular formula the structure of the polyene chain was evident, since 18 carbons and 19 protons together with nine double-bond equivalents still had to be accounted for. One of the carbons was the amide carbon C-1′ (δ_C 171.1), which shows $^1H-^{13}C$ HMBC correlations to H-2′ and H-3′. The resonance signals of most of the carbons and protons of the polyene chain were overlapping, but they clearly showed $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC correlations to each other as well as to the terminal methyl group CH_3-18′ (δ_C 18.5, δ_H 1.82). Thus, the polyene part of **1** had to be 2,4,6,8,10,12,14,16-octadecaoctaenoic acid. An all-*trans* polyene in **1**, as it is also described for the known myxochromides, is in good accordance with the UV spectrum of **1**, which showed a maximum at 422 nm. $^1H-^1H$ coupling constants (^3J, vicinal) can be defined unambiguously only for H-3′ (see Table 1), which also confirms a *trans*-configuration.

Chiral GC-MS was applied to determine the absolute configuration of leucine and glutamine as L, whereas alanine proved to be present in the D- as well as in the L-form. Using spectroscopic methods alone, it is difficult to elucidate the positions of L- and D-alanine within the peptidic moiety of myxochromides. Concerning myxochromide A_2 this question was not solved in the original report of this compound.[5] For the new myxochromide B_3 (**1**), L- and D-alanine were placed according to the suggestion of Wenzel et al.[13,14] for the myxochromides A, which is mainly based on genetic data.

The configuration of proline was investigated by chiral HPLC and determined to be L. Our result is in good accordance with the literature data for myxochromide A_2,[5] which has been reported to consist of L-leucine, D- and L-alanine, L-glutamine, L-proline, and L-*N*-Me-threonine. In analogy with these data, the *N*-Me-threonine of **1** is suggested to also be L-configured.

The structure of compound **2** was deduced using the same methodology as for **1** and found to be identical to that published for myxochromide A_3.[14] Since no spectroscopic data are published for this metabolite, these are included here (see Experimental Section).

Myxochromide B_3 (**1**) and A_3 (**2**) were tested in a disk diffusion assay against *Bacillus megaterium*, *Escherichia coli*, *Eurotium rubrum*, *Microbotryum violaceum*, *Mycotypha microspora*, and *Chlorella fusca* and found to be inactive (concentration 50 μg/disk). They did not show any cytotoxic effects in a panel of 28 tumor cell lines (concentration 10 μg/mL), either.

The structure of myxochromide B_3 (**1**) is of special interest regarding the biosynthesis of this group of natural products. The gene clusters for the myxochromides of the A series (mch_A) as well as the S series (mch_S) from the producer organisms *Stigmatella aurantiaca* DW4/3-1 and *Myxococcus xanthus* DK1050, respectively, have been investigated in detail by the Müller research group.[13,14] Both gene clusters comprise genes encoding nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs), i.e., NRPS−PKS hybrid megasynthetases. According to the colinearity rule, which applies to many biosynthetic gene clusters, the number, nature, and succession of the building blocks can be predicted by the corresponding domains and gene sequences. In the mch_A gene cluster only the alanine-incorporating NRPS module which is placed between the modules responsible for leucine and threonine incorporation was found to contain an epimerization domain.[13,14] From this observation the most probable positions for D- and L-alanine in the peptidic core of myxochromides A could be suggested.

These genetic investigations also revealed that both gene clusters, mch_A and mch_S, are composed of six NRPS modules. Whereas this is in accordance with the hexapeptide structure of the myxochromides of the A series, it is most surprising for the myxochromides of the S series, which are composed of only five amino acids. The mch_S gene cluster contains five modules, which, according to the specifity-conferring sequences of the A domains ("nonribosomal code"[16]), suggest the assembly of exactly those amino acids that are present in the peptidic core and an additional module that does not seem to be active. It is not surprising that the additionally present module has homology to proline-incorporating modules. The biosynthesis of myxochromides S thus probably involves the "skipping" of the proline-incorporating module, resulting in a penta- instead of a hexapeptidic structure.[13]

A further structural difference between myxochromides A and S is the position of the alanine and leucine residues, which was also explained on the genetic level by point mutations.[14] Based on sequence similarities of the corresponding modules in mch_A and mch_S the exchange of complete modules or adenylation domains to explain the difference in amino acid sequence was found to be less likely. The one alanine module of mch_S shows high sequence similarity to the leucine module of mch_A, which is in a corresponding position in the gene cluster. This sequence similarity is higher than that of the two alanine-encoding modules of the mch_S gene cluster, which makes the exchange of whole modules unlikely. This conclusion is further supported by the fact that the leucine-encoding module of the myxochromide S gene cluster still contains the epimerization domain, even though L-leucine is incorporated. Apparently, with the change of selectivity of the adenylation (A) domain the activity of the epimerization (E) domain was lost. In an evolutionary context, these findings suggest the myxochromide A gene cluster to be the original one, which was changed by point mutations to the myxochromide S gene cluster.

In the context of the above discussion on the biosynthesis of myxochromides the finding of myxochromide B_3 (**1**) is most interesting. Presumably, the myxochromide B_3 gene cluster is also derived from the mch_A cluster. The additional leucine residue being located adjacent to the leucine unit present in myxochromides A is intriguing. Thus, for the biosynthesis of myxochromide B_3 two scenarios seem possible: the duplication of the leucine module or the iterative use of only one module. In order to obtain a deeper insight into the evolution of these biosynthetic gene clusters, the investigation of the mch_B cluster would be most interesting.

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 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 500 DRX spectrometer with $(CD_3)_2CO$ or CD_3OD as the solvent and internal standard. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 2.04/29.8 for $(CD_3)_2CO$ and $\delta_{H/C}$ 3.35/49.0 for CD_3OD. ESI-MS were recorded on a micrOTOF spectrometer (Bruker Daltonik, Bremen). 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 used aluminum sheets and silica gel Si 60 F254 (Merck) with the solvent system CH_2Cl_2/MeOH (9:1), detected by quenching of fluorescence at 254 nm and fluorescence at 366 nm and detection with vanillin sulfuric acid reagent and heating to 110 °C.

HPLC was performed using a system consisting of a Waters associated chromatography pump, a Rheodyne 7725i injection system, a Knauer differential refractometer, and a Linseis L200E recorder or a Merck-Hitachi system equipped with a L-6200A pump, a L-4500A photodiode array detector, a D-6000A interface with D-7000 HSM software, and a Rheodyne 7725i injection system. Chiral GC-MS analysis was performed on a Perkin-Elmer Turbomass mass spectrometer using an Alltech Capillary Chirasil-Val column.

Chiral GC-MS. Compound **1** (1.3 mg) was dissolved in 6 N HCl (1 mL) and heated for 16 h at 110 °C in a closed vial. After this hydrolysis, the solvent was removed by a stream of N_2. The dry residue was treated with 500 μL of iPrOH and 150 μL of acetyl chloride at 110 °C for 1 h to give the isopropyl esters of the amino acids. After removal of the solvent, the dry residues were acylated (trifluoroacetic anhydride (400 μL) in CH_2Cl_2 (400 μL); heating for 15 min at 110 °C). The reaction mixture was dried and dissolved in EtOAc (100 μL). A 1 μL aliquot of this solution was analyzed by GC-MS using an Alltech Capillary Chirasil-Val column (25 m × 0.25 mm; 0.16 μm; column temperature kept at 50 °C for 3 min followed by heating from 50 to 180 °C at 4 °C/min; flow 0.6 mL/min and injector temperature 250 °C).

The derivatization of the amino acids was performed using 5−10 mg of D- and L-amino acids and processed as described above. Before being analyzed by GC-MS, the solutions of the standard amino acids were diluted with EtOAc (1:50). The retention times of the standards were D-alanine 11.07 min, L-alanine 11.76 min, D-proline 19.44 min, L-proline 19.48 min, D-leucine 17.06 min, L-leucine 17.88 min, D-glutamine 27.05 min, and L-glutamine 27.37 min.

Retention times of the hydrolysate: 10.99 min (D-alanine), 11.64 min (L-alanine), 17.87 min (L-leucine), 19.53 min (D- or L- proline), and 27.37 min (L-glutamine).

Chiral HPLC. Compound **1** (0.7 mg) was hydrolyzed in 500 μL of 6 N HCl at 110 °C for 16 h. The hydrolysate was dried with a stream of N_2. The residue was dissolved in 120 μL of the mobile phase used for chiral HPLC (2 mM $CuSO_4$ in H_2O/MeCN; 170/9; flow rate 1 mL/min) and centrifuged at 13 000 rpm for 1 min to remove particulates, and then 10 μL of the supernatant was injected. The chiral HPLC was performed using a Chirex 3126 D-penicillamine column (Phenomenex; 250 × 4.60 mm). In this experiment the retention times of the standards were 11.12 min (L-proline) and 20.03 min (D-proline). In the chromatogram of the hydrolysate only one peak appeared, which corresponded to the L-proline retention time. To confirm that this peak belonged to L-proline, the sample was spiked with the reference solution of L-proline, and thus, the configuration of the proline residue was established.

Isolation and Taxonomy of the Bacterial Strain. The soil samples from which the strains no. 163 and no. 171 were isolated had been collected in Brittany, France. 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. Even though both strains lost their ability to form fruiting bodies rather quickly, their typical morphology (slimy knobs) allowed grouping these strains into the genus *Myxococcus*. The myxospores are spherical and the vegetative cells are slender rods, which is in good accordance with the classification. The result of the 16S rDNA sequencing analysis for

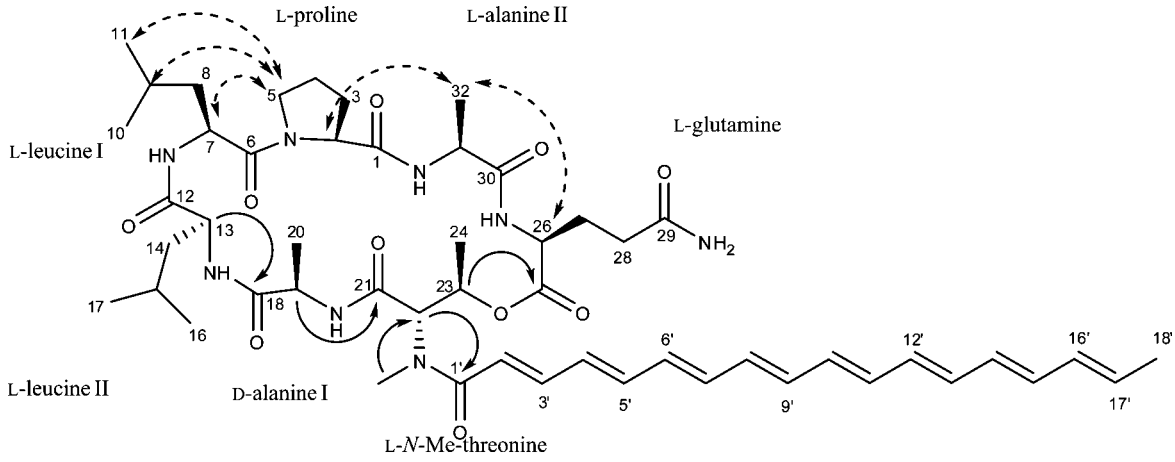


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Figure 2. Selected HMBC (solid) and ROESY (dashed) correlations which helped to establish the amino acid sequence of myxochromide B_3 (**1**).

strain 171 supports these findings. Stock cultures of the strains were kept at −80 °C.

Isolation Procedure. Cultivation was performed in six 5 L Erlenmeyer flasks, each containing 1.5 L of a peptone medium with 2% Amberlite XAD-16 (Fluka, Germany). The medium consisted of Casiton 3 g/L, $CaCl_2 \cdot 2H_2O$ 0.7 g/L, $MgSO_4 \cdot 7H_2O$ 2 g/L, yeast extract 3 g/L, starch 10 g/L, and glucose·H_2O 1 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 7 days. At the end of the cultivation, the bacterial cells and the adsorber resin were separated from the culture broth by centrifugation and extracted 5× with acetone (each time applying 200 mL). After removal of the solvent under reduced pressure (ca. 40 °C), the residue (3.94 g) was suspended in 60% aqueous MeOH (100 mL) and extracted 5× with CH_2Cl_2 (100 mL). The CH_2Cl_2 layers were combined and dried (1.34 g). Separation of this extract by column chromatography over Si gel (Merck, 63−200 μm; approximately 30 g) by consecutively employing CH_2Cl_2, EtOAc, acetone, and MeOH as eluents (100 mL of each solvent) gave four fractions. TLC guided, the third fraction (475 mg) was chosen for further separation and subjected to RP-VLC with MeOH/H_2O (50/50, 100 mL), MeOH/H_2O (70/30, 100 mL), and acetone as the eluting solvents. Semipreparative HPLC (column: Eurospher-100, Knauer, 5 μm, 250 × 8 mm, eluent: petroleum ether/acetone (50/50), flow rate: 2 mL/min) gave 2 mg of nearly pure **1** at a retention time of 13.8 min.

Compound **2** was also isolated from a 9 L culture (6 × 1.5 L) in the presence of Amberlite XAD-16, which was extracted with 1400 mL of acetone to give 3.28 g of crude extract. Analogous to the isolation of compound **1** the crude extract was partitioned and the CH_2Cl_2 fraction separated by VLC, which yielded 373 mg of the myxochromide-containing acetone fraction. This fraction was subjected to a further normal-phase VLC with petroleum ether/acetone (80/20, 75 mL), petroleum ether/acetone (70/30, 50 mL), and acetone (50 mL) as the eluting solvents. Semipreparative reversed-phase HPLC (column: Eurospher-100, C-18, Knauer, 5 μm, 250 × 8 mm) of the acetone fraction with MeOH/H_2O (90/10) as the eluent and a flow rate of 2 mL/min gave 7 mg of **2** at a retention time of 12.5 min.

Myxochromide B_3 (1): yellow solid; $[\alpha]^{20}_D$ −56.6 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 422 (4.77) nm; IR ν_{max} 3315, 2925, 1631, 1530, 1444, 1385, 1365, 1281, 1228, 1103, 1009, 871 cm^{-1}; for ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m/z* 981.5418 (calcd for $C_{51}H_{74}N_8O_{10}Na$ 981.5420).

Myxochromide A_3 (2): yellow solid; $[\alpha]^{20}_D$ −7.96 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 406 (4.12) nm; IR ν_{max} 3312, 2924, 1632, 1531,1445, 1393, 1276, 1104, 1008, 874 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2CO$, reference δ = 2.04 ppm) δ 7.98 (1H, d, *J* = 9.8, 20-NH), 7.94 (1H, d, *J* = 7.6, 25-NH), 7.80 (1H, d, *J* = 7.3, 7-NH), 7.28 (1H, dd, *J* = 11.6, 14.5, H-3′), 7.09 (1H, d, *J* = 9.5, 13-NH), 6.95 (2H, s, 23-NH_2), 6.55 (1H, H-2′), 6.48 (1H, m), 6.72−6.55 (11H, m, H-5′−15′), 6.13 (1H, m, H-16′), 5.76 (1H, m, H-17′), 5.66 (1H, m, H-17), 5.57 (1H, d, *J* = 3.2, H-16), 4.69 (1H, m, H-13), 4.52 (1H, m, H-20), 4.51 (1H, m, H-7), 4.09 (1H, t, *J* = 8.5, H-2), 3.83 (1H, q, *J* = 7.3, H-25), 3.77 (1H, m, H-5a), 3.58 (1H, m, H-5b), 3.30 (3H, s, 16-*N*-CH_3), 2.34 (1H, m, H-21a), 2.32 (1H, m, H-22a), 2.20 (1H, m, H-3a), 2.18 (1H, m, H-22b), 2.14 (1H, m, H-4a), 1.99 (1H, m, H-4b), 1.87 (1H, m, H-3b), 1.84 (1H, m, H-9), 1.76 (3H, d, *J* = 6.6, H-18′), 1.69 (1H, m, H-21b), 1.55 (1H, m, H-8a), 1.47 (3H, d, *J* = 7.3, H-26), 1.44 (1H, m, H-8b), 1.15 (3H, d, *J* = 6.6, H-14), 1.05 (3H, d, *J* = 6.6, H-18), 0.95 (3H, d, *J* = 6.6, H-11), 0.93 (3H, d, *J* = 6.6, H-10); ^{13}C NMR (75 MHz, $(CD_3)_2CO$, reference: δ = 29.8 ppm) δ 175.6 (C, C-19), 173.4 (C, C-12), 172.4 (C, C-1), 172.3 (C, C-23), 171.3 (C, C-24), 171.2 (C, C-6), 168.6 (C, C-1′), 168.5 (C, C-15), 143.6 (CH, C-3′), 140.7−133.3 (CH, C-5′−15′), 133.0 (CH, C-16′), 131.2 (CH, C-17′), 131.6 (CH-C-4′), 121.0 (CH, C-2′), 71.9 (CH, C-17), 63.2 (CH, C-2), 60.1 (CH, C-16), 53.4 (CH, C-20), 51.6 (CH, C-25), 51.1 (CH, C-7), 48.3 (CH, C-13), 47.6 (CH_2, C-5), 38.8 (CH_2, C-8), 34.9 (CH_3, 16-*N*-CH_3), 32.2 (CH_2, C-22), 29.8 (CH_2, C-21), 29.7 (CH_2, C-3), 26.3 (CH_2, C-4), 25.8 (CH, C-9), 23.7 (CH_3, C-10), 21.1 (CH_3, C-11), 18.5 (CH_3, C-18′), 16.8 (CH_3, C-18), 16.7 (CH_3, C-14), 16.2 (CH_3, C-26); HRESIMS *m/z* 868.4582 (calcd for $C_{45}H_{63}N_7O_9Na$ 868.4579).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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