Isolation of Aerucyclamides C and D and Structure Revision of Microcyclamide 7806A: Heterocyclic Ribosomal Peptides from *Microcystis aeruginosa* PCC 7806 and Their Antiparasite Evaluation

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Aerucyclamides C and D were isolated from the cyanobacterium *Microcystis aeruginosa* PCC 7806, and their structures established by NMR spectroscopy and chemical transformation and degradation. Acidic hydrolysis of aerucyclamide C (CF_3CO_2H, H_2O) resulted in microcyclamide 7806A. This chemical evidence combined with spectroscopic and physical data suggest a structure revision for microcyclamide 7806A, which incorporates an O-acylated Thr ammonium residue instead of the originally proposed methyl oxazoline ring. We have prepared microcyclamide 7806B upon basic and acidic treatment of microcyclamide 7806A, which suggests that both these compounds are hydrolysis products of aerucyclamide C and that the aerucyclamides A−D are the actual metabolites produced via ribosomal peptide synthesis in *M. aeruginosa* PCC 7806. Antiplasmodial evaluation established submicromolar IC_{50} values for aerucyclamide B against *Plasmodium falciparum*; low micromolar values for aerucyclamide C were found against *Trypanosoma brucei rhodesiense*. The compounds were selective for the parasites over a cell line of L6 rat myoblasts and are thus considered for further study as antimalarial agents.

Cyanobacteria are prolific producers of novel bioactive metabolites,[1] and in particular the structural diversity of the peptides produced is remarkable.[2] Certain cyclic peptides involving heterocyclic modifications (sometimes called cyclamides) occur in several marine animals[3] and in cyanobacteria.[4] The latter species are considered the true source of these metabolites, as the sea hares and ascidians receive these compounds by cyanobacterial diet or from cyanobacterial symbionts. Typically, such cyclamides are found accumulated in higher trophic levels, and this compound class encompasses hexa- and octameric cyclopeptides alternating in hydrophobic and hydrophilic (Ser, Thr, and Cys) amino acids.[3,4] The side chains of these polar amino acids can be heterocyclized to form oxazole or thiazole rings or their reduced derivatives, thus resulting in rather planar, disk-like structures.[4c,5] Despite their widespread occurrence, the ecological function of these compounds remains unclear. Several groups recently investigated the biosynthesis of these and similar heterocyclic cyclopeptides, and ribosomal peptide synthesis was established for the patellamide family of natural products.[6] In this biosynthetic scheme, hypervariable cassettes, encoding ribosomally for the cyclopeptides, are flanked by conserved genes, which contain the genetic information for the processing enzymes such as proteases, cyclases, and oxidases. In the cyanobacterial genus *Microcystis*, a similar pathway was recently discovered,[7] and this genetic information was used to predict metabolites present in *Microcystis aeruginosa* PCC 7806. On the basis of this genetic information, Dittmann and co-workers isolated microcyclamide 7806A and microcyclamide 7806B and assigned the structures **1** and **3**, respectively. Independently, we isolated from the same strain the aerucyclamides A (**4**) and B (**5**), which displayed moderate grazer toxicity against the crustacean *Thamnocephalus platyurus*.[8] In this publication, we report the isolation and structure elucidation of the remaining members of this family, aerucylamides C (**6**) and D (**7**), from *M. aeruginosa* PCC 7806. In addition, we provide physical, chemical, and

spectroscopic evidence for a revised structure of microcyclamide 7806A (revised structure, **2**). Antiparasite evaluation of the aerucyclamides **4**−**7** demonstrated submicromolar activity and selectivity against *Plasmodium falciparum* for aerucyclamide B (**6**).

Results and Discussion

Structure of Aerucyclamide C (6). The molecular formula ($C_{24}H_{32}N_6O_5S$) of aerucyclamide C (**6**) was suggested by high-resolution MS, which was complemented by isotope labeling studies, establishing the presence of six N atoms and one S atom. The ^{1}H NMR spectrum displayed the pattern of a peptide. On comparison of its NMR data with aerucyclamides A (**4**) and B (**5**), several observations were made. While the typical signals of the MeOzn were still present, in the aromatic region, two aromatic singlets were observed. The two singlet signals were attributed to an oxazole (Ozl) and a thiazole (Tzl) ring according to their chemical shifts: The resonance at δ 8.42 was attributed to the thiazole H-13 and the downfield singlet (δ 8.81) was attributed to the oxazole H-19.[3f] The other amino acids were designated as Ala, Val, and Ile based on typical signals. This was corroborated by 2D-NMR experiments, where COSY and TOCSY allowed for the assignment of resonances in the ^{1}H NMR spectrum and HSQC and HMBC allowed for the assignment of ^{13}C resonances. The sequence was clearly established by HMBC long-range correlations. Correlations were observed from H-2 to C-5, from H-6 to C-8, from H-10 to C-11, from H-12 Ile to C-17, from H-19 to C-20, and from H-21 Val to C-1 (Figure 1). Only one connection between the two carbon atoms C-17 and C-18 could not be established based on the HMBC correlations, but according to the molecular formula, aerucyclamide C (**6**) was required to be tetracyclic and therefore C-17 and C-18 needed to be connected, thus closing the macrocyclic ring. As for other members of this family, the typical long-range *J* coupling (2.1 Hz) between the C(α)-H of the MeOzn and the Ala residue was observed.[3a,d,4a,8]
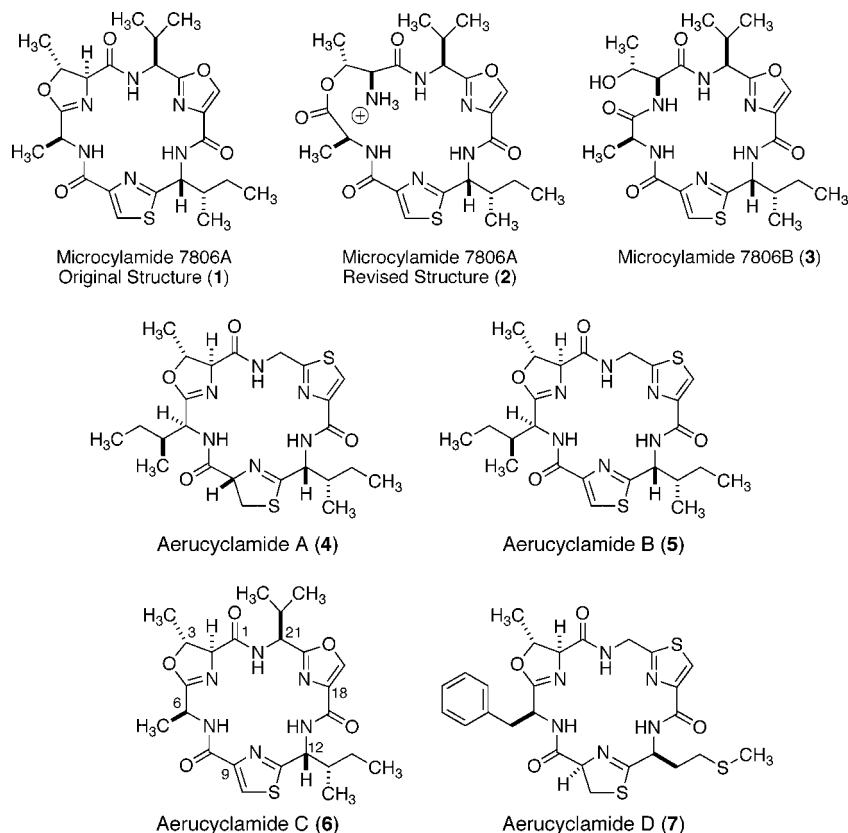
The configuration of aerucyclamide C was assigned after chemical degradation, derivatization of the resulting amino acids, and analysis by GC and HPLC using chiral stationary phases or Marfey's method,[9] respectively. Aerucyclamide C (**6**) was flash hydrolyzed (6 N HCl solution, 105 °C, 90 min), and a mixture of

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Microcylamide 7806A
Original Structure (**1**)

Microcylamide 7806A
Revised Structure (**2**)

Microcylamide 7806B (**3**)

Aerucyclamide A (**4**)

Aerucyclamide B (**5**)

Aerucyclamide C (**6**)

Aerucyclamide D (**7**)

D-*allo*-Ile and L-Ile was detected by GC-MS on chiral stationary-phase columns. It is known in the literature that amino acids next to thiazole or oxazole rings are prone to epimerization during hydrolysis.[10] To avoid the epimerization of the Ile residue, compound **6** was first ozonized prior to the flash hydrolysis and derivatization with Marfey's reagent. This method, combined with the GC-MS analysis described above, thus clearly established the Ile residue to be of D-*allo*-Ile configuration. The other stereogenic centers were assigned after ozonolysis, hydrolysis, and Marfey derivatization as L-Ala, L-Val, and L-Thr residues. These experiments thus establish the structure of aerucyclamide C as shown for **6**.

Structure of Aerucyclamide D (7). The high-resolution mass spectrum of aerucyclamide D (**7**) displayed an exact mass of *m/z* 587.1588, which supports the molecular formula $C_{26}H_{31}N_6O_4S_3$ (calcd. 587.1569) for the proton adduct. The ^1H NMR spectrum (DMSO-d_6, 600 MHz) displayed the typical pattern of a peptide. All spin systems could be assigned by COSY and TOCSY experiments, and ^{13}C chemical shifts were assigned by HSQC and HMBC experiments. Three NH resonances were present, two of which appeared as a doublet and one as a multiplet. The first two NH doublets were attributed to the Phe and Met residues based on

Aerucyclamide C (**6**)

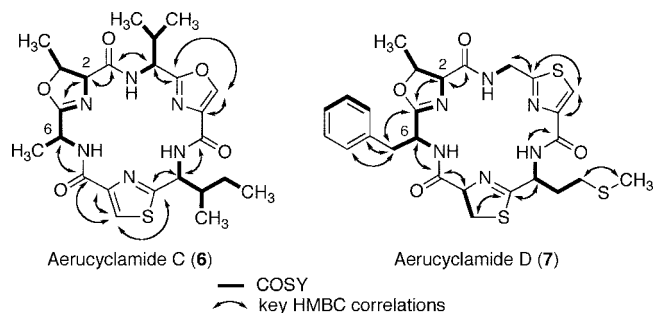Aerucyclamide D (**7**)

— COSY
⌢ key HMBC correlations

Figure 1. Key COSY and HMBC correlations in aerucyclamides C (**6**) and D (**7**). Note the observed long-range *J* coupling between H-2 and H-6 in both aerucyclamides C (**6**) and D (**7**).

typical signals and the COSY and TOCSY spectra, and the multiplet NH was attributed as a Gly residue. Characteristic signals pointed out the presence of a methyloxazoline (MeOzn) fragment as for the other aerucyclamides. These two-dimensional NMR data corroborated the presence of NH-Gly, NH-Phe, NH-Met, and the MeOzn fragments. Additionally, a CH_2 of a thiazoline residue (based on the chemical formula) and a sharp aromatic singlet were observed. The remaining two amino acids of this cyclic hexapeptide were thus assigned as thiazoline (Tzn) and thiazole (Tzl) residues, based on the presence of two sulfur atoms and characteristic signals. The sequence was clearly established by HMBC long-range correlations. Correlations were observed from H-2 to C-5, from H-6 to C-12, from H-14 to C-15, from NH of Met to C-20, and from H-22 to C-23 (Figure 1). Two correlations between NH of Gly and C-1 of MeOzn and between C-20 and C-21 could not be observed. These connections were assigned on the basis that the peptide was pentacyclic according to the molecular formula. It is interesting to note that again the typical long-range *J* coupling (1.8 Hz) between the C(α)-H of the MeOzn and the Phe residue could be observed. This coupling is frequently present in similar cyclamides with oxazoline ring systems.[3a,d,4a,8] The stereogenic centers were assigned by similar strategies to those described above. Hydrolysis and Marfey derivatization[9] of aerucyclamide D (**7**) established the presence of L-Cys, L-Phe, and L-Thr. L-Met was assigned after ozonolysis prior to the hydrolysis and subsequent Marfey derivatization. These data thus establish the structural assignment of aerucyclamide D to be as shown for compound **7**.

Structure Revision of Microcylamide 7806A. The structural identity of aerucyclamide C (**6**) and microcylamide 7806A (original structure, **1**) based on different spectroscopic data (this work and ref 7) prompted us to re-evaluate the structural assignment of microcylamide 7806A. Inspection of the data reported by Dittmann and co-workers[7] presents several inconsistencies with the originally proposed structure **1** of microcylamide 7806A. While the lipophilic aerucyclamides A–D elute late on a reversed-phase C_{18} column, the microcylamides 7806A and B elute earlier and are thus more polar. Both microcylamides 7806A and B elute before several other

Table 1. NMR Spectroscopic Data (600 MHz, DMSO-d_6) for Aerucyclamide C (**6**)

C/H no.	δ_C	δ_H (J in Hz)	HMBC[a]
1	169.8, qC		
2	73.3, CH	4.36, dd (2.1, 8.1)	1, 3, 4, 5
3	82.4, CH	4.81, dq (6.4, 8.1)	1, 5
4	21.5, CH$_3$	1.50, d (6.4)	3
5	169.4, qC		
6	43.8, CH	4.72, ddq (2.1, 6.2, 7.0)	5, 7, 8
7	18.5, CH$_3$	1.44, d (7.0)	5, 6
NH (2)		8.60, d (6.2)	5, 6, 8
8	158.7, qC		
9	147.1, qC		
10	125.6, CH	8.42, s	8, 9, 11
11	169.0, qC		
12	54.0, CH	5.54, dd (3.3, 8.0)	11, 13, 14, 17
13	42.0, CH	1.96, m	11, 12
14	13.9, CH$_3$	0.81, d (6.8)	12, 13, 15
15	25.4, CH$_2$	1.55, m	12, 13, 14, 16
		0.92, m	12, 13
16	11.8, CH$_3$	0.91, dd (6.8, 7.3)	
NH (4)		8.53, d (8.0)	11, 12, 17
17	158.5, qC		
18	134.4, qC		
19	143.0, CH	8.81, s	18, 20
20	162.9, qC		
21	52.3, CH	5.08, dd (3.6, 7.8)	1, 20, 22, 23, 23′
22	32.1, CH	2.29, m	20, 21
23	17.3, CH$_3$	0.81, d (6.9)	21, 22
23′	18.1, CH$_3$	0.81, d (6.9)	21, 22
NH (6)		7.94, d (7.8)	1, 20, 21

[a] HMBC correlations are given from the proton(s) stated to the indicated carbon.

Table 2. NMR Spectroscopic Data (600 MHz, DMSO-d_6) for Aerucyclamide D (**7**)

C/H no.	δ_C	δ_H (J in Hz)	HMBC[a]
1	169.2, qC		
2	72.8, CH	4.23, dd (7.2, 1.8)	1, 4, 5
3	80.4, CH	4.95, m	5
4	21.1, CH$_3$	1.45, d (6.2)	2, 3
5	166.2		
6	47.2, CH	4.94, m	12
7	36.1, CH$_2$	2.96, d (4.1)	5, 6, 8, 9/9′
8	135.2, qC		
9/9′	129.1, CH	6.72, d (7.2)	7, 9/9′, 11
10/10′	127.0, CH	6.68, dd (7.2, 7.4)	8, 10/10′
11	125.7, CH	6.38, t (7.4)	9/9′
NH (2)		7.21, d (8.3)	12
12	168.9, qC		
13	77.5, CH	5.24, dd (9.4, 9.4)	12, 14, 15
14	34.8, CH$_2$	3.77, dd (11.0, 9.4)	12, 13
		3.71, dd (11.0, 9.4)	12, 13, 15
15	172.1, qC		
16	50.2, CH	5.07, dd (12.6, 5.9)	15, 17, 18
17	33.7, CH$_2$	2.15, m	19
		1.94, m	19
18	28.0, CH$_2$	2.41, m	
19	14.4, CH$_3$	2.02, s	18
NH (4)		7.80, d (7.3)	15, 20
20	159.3, qC		
21	147.9, qC		
22	125.0, CH	8.35, s	21, 23
23	165.7, qC		
24	40.0, CH$_2$	4.71, dd (17.8, 5.1)	23
		4.41, dd (17.8, 3.5)	23
NH (6)		7.31, m	

[a] HMBC correlations are given from the proton(s) stated to the indicated carbon.

peptides present in *M. aeruginosa*, the microcystins and the cyanopeptolins. In addition to this physical difference of polarity, NMR chemical shift data were reported[7] that are unlikely to match with the proposed structure **1**. Inspection of the NMR data reported for microcyclamide 7806A[7] and comparison to ¹³C chemical shift data reported for similar oxazoline-containing peptides such as the aerucyclamides A−D,[8] didmolamide,[3f] bistratamides A,[3a] E, and F,[3e] and banyascyclamide A[4i] revealed rather large differences at carbon atoms 1−5 (see Table 3). While the chemical shifts of the C-2 carbon of the oxazoline ring usually appear between 72.6 and 74.1 ppm, the corresponding value in microcyclamide 7806A was observed at 55 ppm (over 17 ppm difference). A similar situation is observed for the resonance of the C-3 atom. While literature values range from 79 to 82.1 ppm, the reported value for microcyclamide 7806A was 69.7 ppm. Lastly, the reported values for C-1, C-4, and C-5, as well as the relevant protons, of microcyclamide 7806A significantly differ from literature values of similar oxazoline-containing peptides. These values of the physical and spectroscopic data cast doubt on the presence of an oxazoline ring in microcyclamide 7806A.

We have obtained a sample of microcylamide 7806A by treating a sample of aerucyclamide C (**6**) with 1% trifluoroacetic acid in H₂O for 12 h (Scheme 1). The RP-HPLC profile of the synthetic sample of microcystin 7806A matches the one reported by Dittmann and co-workers with respect to the retention time, as this sample elutes before the microcystin and cyanopeptolin fractions of *M. aeruginosa* PCC 7806. In addition, we fully characterized the synthetic sample of microcyclamide 7806A by NMR spectroscopy, and the measured values match those reported by Dittmann and co-workers, with one exception. We were able to detect a broad signal at 8.28 ppm. This exchangeable signal displays a COSY correlation to H-2. We thus assign this signal to the presence of the NH₃ ammonium group of the Thr residue. This assignment is also supported by the chemical shifts, which display values expected for an O-acylated Thr ammonium residue upon comparison to literature values.[11] In particular, Murakami and co-workers reported hydrolysis products of the cyanobacterial siderophore

anachelin,[11b−11d] where the oxazoline ring was opened to an ammonium ester.[11c] The chemical shifts of the respective carbon atoms in the ammonium ester derivative of anachelin are similar to those of microcyclamide 7806A.

The chemical evidence that microcylamide 7806A constitutes a hydrolysis product (after acidic treatment) of aerucyclamide C (**6**) combined with the spectroscopic data of both chemical shifts and the presence of a broad NH₃ signal suggest that the revised structure **2** is the actual structure of microcyclamide 7806A. This is further corroborated by the measured HRESIMS data of microcyclamide 7806A, which were consistent with the molecular formula C₂₄H₃₅N₆O₆S for the proton adduct, which also supports the revised structure **2** (see Experimental Section). We have also observed a small peak ($m/z = 517$) in the mass spectrum of **2** that can be assigned to the proton adduct of a dehydrated structure identical or isomeric to **1**. In addition, we were able to convert microcyclamide 7806A to the isomer microcyclamide 7806B[7] (**3**) by exposure to NaOD and subsequent neutralization with aqueous DCl solution. These lines of evidence corroborate that both microcyclamide 7806A (**2**) and microcyclamide 7806B (**3**) reported by Dittmann and co-workers are hydrolysis products of aerucyclamide C (**6**), which is supported by their reported isolation procedure that employed trifluoroacetic acid.[7] We did not directly observe microcyclamide 7806A and B in extracts of *M. aeruginosa* PCC 7806 because we avoided acidic conditions and assume that both were generated during the isolation procedure employed.[7] As a last point, there are inconsistencies in the configurational assignment of the Ile of microcyclamide as reported by Dittmann and co-workers.[7] While in the Experimental Section the presence of D-Ile is reported, D-*allo*-Ile is drawn in their publication, and both epimers are discussed in the text.[7] The data presented in this publication clearly assign the D-*allo*-Ile configuration to this residue in microcyclamide 7806A (**2**) as well as aerucyclamide C (**6**). Lastly, significant differences in the biological properties of the microcyclamides 7806A and B[7] and other cyclamides[3,4,8] are worth commenting on. While for the microcyclamides 7806A and B no activity in

Table 3. Comparison of the Reported ^{13}C NMR Chemical Shift Data (in ppm) for the Methyl Oxazoline Fragment of Microcyclamide 7806A[7] and Other Methyl Oxazoline-Containing Cyclamides
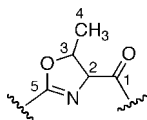
C atom	microcyclamide 7806A[7]	aerucyclamide A[8]	aerucyclamide B[8]	banyascyclamide A[4i]	didmolamide B[3f]	bistratamide A[3a]	bistratamide E[3e]	bistratamide F[3e]
1	166.2	169.2	169.0	169.3	169	170.2	169	169.4
2	55	72.6	72.2	73.9	74.1	73.9	72.7	72.7
3	69.7	79	79.8	81.9	81.9	80.8	81.9	81.7
4	17.1	20.5	20.3	21.7	22.9	21.4	21.4	21.4
5	171.3	166.1	166.3	167.6	170.8	168.4	167.6	167.2

Scheme 1. Hydrolysis of Aerucyclamide C (**6**) to Microcyclamide 7806A (**2**) and Microcyclamide 7806B[7] (**3**)

Aerucyclamide C (**6**)

1% CF$_3$CO$_2$H, H$_2$O
RT, 12 h

Microcylamide 7806A
Revised Structure (**2**)

aq. base and acid
equilibration

Microcylamide 7806B (**3**)

Table 4. Biological Evaluation of Aerucyclamides **4**−**7** against *Plasmodium falciparum* K1, *Trypanosoma brucei rhodesiense* STIB 900, and Rat Myoblast L6 Cells (IC$_{50}$ values are reported as μM concentrations)

	P. f.[a]	*T. b. rhod*[b]	L6[c]
4	5.0	56.3	>168
5	0.7	15.9	120
6	2.3	9.2	106
7	6.3	50.1	>153

[a] *Plasmodium falciparum* K1. [b] *Trypanosoma brucei rhodesiense* STIB 900. [c] Rat myoblast L6 cells.

clamides also point out that the "prediction of metabolites from genetic data"[7] is still challenged by such structural issues at present.

In the context of our search for lead structures against tropical infectious diseases from cyanobacteria,[12] we tested the aerucyclamides C (**6**) and D (**7**), together with the congeners **4** and **5**, for antiparasite activity. Recently, Gerwick and co-workers have shown that similar cyclamides, the venturamides A and B, display antiplasmodial activity.[4j] Venturamide A displayed an IC$_{50}$ value of 8.2 μM against strain W2 of *P. falciparum*, and venturamide B an IC$_{50}$ of 5.2 μM. Cytotoxicity values were determined to be 13.1 and >54 μM, respectively, against MCF-7 cancer cell lines, which can be considered of moderate selectivity. This initial report thus encouraged us to further evaluate cyclamide derivatives for antiplasmodial activity, and the results for compounds **4**−**7** are given in Table 4.

The most active compound was aerucyclamide B (**5**), displaying a submicromolar IC$_{50}$ value against the chloroquine-resistant strain K1 of *P. falciparum*. In addition, this compound displays a large selectivity for the parasite with respect to the L6 rat myoblast cell line, where an IC$_{50}$ value of 120 μM was determined. Interestingly, the antiplasmodial activity is decreased by 1 order of magnitude by one structural modification from **5** to **4**, i.e., reduction of the thiazole to a thiazoline residue. Similar activities were determined for aerucyclamide D (**7**) and aerucyclamide C (**6**), which displayed low micromolar activity. All compounds displayed very weak (for **5** and **6**) or no toxicity to the L6 cell line under the conditions evaluated. Against *T. brucei rhodesiense*, the most active compound was aerucyclamide C (**6**), albeit with moderate activity. Compound **5** and, in particular, compounds **4** and **7** were clearly less active against this parasite. Overall, these results demonstrate that aerucyclamides and, in particular, aerucyclamide B (**5**) display rather potent and selective antiplasmodial activity against *P. falciparum* K1 and a large selectivity over the mammalian L6 cell line. This study thus complements earlier work[4j] and suggests further investigations of these compounds in animal models. We have also tested aerucyclamide C (**6**) for toxicity against the crustacean grazer *T. platyurus*, but found only low toxicity (LC$_{50}$ value 70.5 μM).

In conclusion, we have reported the isolation and structure determination of aerucyclamides C (**6**) and D (**7**), heterocyclic cyclopeptides from the cyanobacterium *M. aeruginosa* PCC 7806. These peptides have been predicted by an earlier study to be products of ribosomal peptide synthesis.[7] In addition, we provide

antiproliferative, antifungal, antibacterial, and cytotoxic assays was observed,[7] cyclamides generally display a wealth of different bioactivities[3,4,8] (also *vide infra*). It is thus reasonable to speculate that the ring-opened hydrolysis products such as **2** and **3** might lose their activity, when compared to the structurally rigid, disklike cyclamides such as **4**−**7**.

In addition, this study proposes that the aerucyclamides A−D (**4**−**7**) are the actual, ribosomally encoded metabolites, in line with the genetic data reported by Dittmann and co-workers.[7] It is interesting to note that there are four genetic sequences found (ATVSIC, FTGCMC, ITGCIC, ITGCIC) that encode for the four aerucyclamides. In particular the presence of the latter two identical sequences raises the question whether aerucyclamide B is produced from aerucyclamide A via oxidation or via a distinct biochemical pathway. The presence of epimerized amino acids as well as the different oxidation states of the heterocyclic rings in the aerucy-

several lines of chemical, physical, and spectroscopic evidence for a revised structure of microcyclamide 7806A (**2**), which should thus be considered as a hydrolysis product of aerucyclamide C (**6**). In addition, it has been shown that acidic and basic treatment of solutions of **2** led to microcyclamide 7806B (**3**). Biological evaluation of aerucyclamides A–D for antiplasmodial activity established that aerucyclamide B (**5**) displayed submicromolar activity against *P. falciparum* K1, with selectivity against a rat myoblast cell line. This study thus suggests that aerucyclamides A–D are the actual metabolites produced by ribosomal peptide synthesis in *M. aeruginosa* PCC 7806 and lays the foundation for their further evaluation as antimalarial agents.

Experimental Section

General Experimental Procedures. NMR spectra were acquired on a Bruker DRX-600 equipped with a cryoprobe and referred to residual solvent proton and carbon signals (δ_H 2.50, δ_C 39.5 for DMSO-d_6). Accurate mass ESI spectra were recorded on a Micromass (ESI) Q-TOF Ultima API. HPLC purification and analyses were performed on a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, a TCC-100 thermostated column compartment, a PDA-100 photodiode array detector, a Foxy Jr. fraction collector, and a MSQ-ESI mass spectrometric detector. The determination of the Ile enantiomers was carried out on a GC-MS instrument (Fison Instruments, GC 8000 Top, MD 800).

Culture, Extraction, and Isolation. *M. aeruginosa* PCC 7806 was obtained from the Pasteur Culture Collection of Cyanobacteria, Paris, France, and cultured as previously described.[8] Freshly thawed biomass was extracted three times with 60% aqueous MeOH; the extract was separated from the biomass by centrifugation. MeOH was removed from the combined extract by evaporation under reduced pressure; the mixture was then dried by lyophilization. The resulting powder was dissolved in 80% aqueous MeOH and centrifuged to remove remaining particles. The solution was then sequentially subjected to a C_{18} SPE column (5 g, conditioned with 10% MeOH), and the compounds were eluted with 10%, 60%, 80%, and 100% MeOH. The combined 100% MeOH fractions were dried under reduced pressure. The resulting residue was dissolved in 70% MeOH, and the compounds were isolated using multiple C_{18} RP-HPLC runs (Phenomenex Gemini C_{18} 5 μm; 150 × 10 mm). The gradient CH_3CN/H_2O at a flow of 5 mL/min was the following: 30% CH_3CN to 35% over 10 min and 35% to 60% from 10 to 30 min. The column was then washed for 10 min with 100% CH_3CN and stabilized for the next cycle in 10 min with 30% CH_3CN. Aerucyclamides C (**6**) and D (**7**) eluted at 21.2 and 14.1 min with 0.3% and 0.2% yield from crude extract, respectively.

Aerucyclamide C (6): colorless, amorphous solid; UV (CH_3CN/H_2O 50/50 v/v) λ_{max} 203, 219; ^{1}H and ^{13}C NMR data (DMSO-d_6), see Table 1; HRESI QqTof-MS *m/z* [M + H]$^+$ 517.2237 (calcd for $C_{24}H_{33}N_6O_5S$, 517.2233).

Aerucyclamide D (7): colorless, amorphous solid; UV (CH_3CN/H_2O 42/58) λ_{max} 240; ^{1}H and ^{13}C NMR data (DMSO-d_6), see Table 2; HRESI QqTof-MS *m/z* [M + H]$^+$ 587.1588 (calcd for $C_{26}H_{31}N_6O_4S_3$, 587.1569).

Configuration of 6 and 7. The hydrolysis, ozonolysis, TFA derivatization, and the Marfey analysis were performed according to procedures previously described.[8] Retention times (in minutes) for the standard amino acid Marfey derivatives were the following: L-Ala 31.3; D-Ala 35.7; L-Val 39.6; D-Val 47.1; L-Thr 26.4; L-*allo*-Thr 27.0; D-Thr 32.1; D-*allo*-Thr 30.0; L-Ile 44.7; L-*allo*-Ile 45.6; D-Ile 52.2; D-*allo*-Ile 52.1; L-Cys (di-FDAA derivative) 45.7; D-Cys (di-FDAA derivative) 49; L-Phe 45.1; D-Phe 50.5; L-Met 38.5; D-Met 44.5.

Aerucyclamide C (**6**) from hydrolysis–TFA on GC-MS: L-Ile or D-*allo*-Ile; from ozonolysis–hydrolysis–Marfey on HPLC: D-Ile or D-*allo*-Ile, L-Ala, L-Val, L-Thr (for L-Thr a co-injection with L-*allo*-Thr was carried out); see also Supporting Information.

Aerucyclamide D (**7**): from hydrolysis–Marfey L-Cys, L-Phe, L-Thr (for L-Thr a co-injection with L-*allo*-Thr was carried out); from ozonolysis–hydrolysis–Marfey L-Met (see also Supporting Information).

Hydrolysis of 6 to 2. Aerucyclamide C (**6**, 0.9 mg, 1.74 μmol) was stirred in 1% CF_3CO_2H in H_2O (1 mL) for 12 h at RT. The reaction mixture was analyzed by HPLC, indicating complete conversion. Microcyclamide 7806A (**2**) was isolated using multiple C_{18} RP-HPLC runs (Phenomenex Gemini C_{18} 5 μm; 150 × 10 mm). The gradient employed (CH_3CN 0.05% TFA (**A**)/H_2O 0.05% TFA (**B**)) at a flow of 5 mL/min was the following: 10% **A** to 55% over 15 min; the column was then washed for 10 min with 100% **A** and stabilized for the next cycle in 10 min with 10% **A**. Compound **2** eluated at 10.2 min. The combined fractions were lyophilized to afford 0.5 mg of microcyclamide 7806A (**2**). See Supporting Information for HPLC data.

Microcyclamide 7806A (2): colorless, amorphous solid; UV (CH_3CN 0.05% TFA/H_2O 0.05% TFA 41/59) λ_{max} 207; ^{1}H and ^{13}C NMR data (DMSO-d_6), see Supporting Information; HRESI QqTof-MS *m/z* [M + H]$^+$ 535.2318 (calcd for $C_{24}H_{35}N_6O_6S$, 535.2339).

Conversion of 2 to 3. In an NMR tube, microcyclamide 7806A (**2**, 0.5 mg, 9.5 μmol) in DMSO-d_6 was treated with 30% NaOD in D_2O (3 μL) and then neutralized with 20% DCl in D_2O (2 μL) to afford microcyclamide 7806B (**3**). The ^{1}H NMR data of compound **3** matched the values reported in the literature[7] (see also Supporting Information).

Twenty-four-hour Acute Grazer Toxicity Bioassay. Aerucyclamide C (**6**) was tested in a 24 h acute toxicity assay performed with *Thamnocephalus patyurus* (Thamnotoxkit F; G Persoone, State University of Ghent, Belgium). The assay was performed in a multiwell plate using instar II–III larvae hatched from cysts. Six different concentrations of **6** ranging from 1 to 100 μM were tested in triplicate. The nonlinear regression analysis was done with Graph Pad Prism, Version 4.0 (Graph Pad Software, Inc.; San Diego, CA).

***Plasmodium falciparum* in Vitro Assay.** Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay was used.[13] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi [^{3}H]-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled H_2O. The radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves IC_{50} values were calculated. Assays were run in duplicate and repeated once.

***Trypanosoma b. rhodesiense* and Cytotoxicity *in Vitro* Assay.** Minimum essential medium (50 μL) supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum[14] was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then 10^4 bloodstream forms of *Trypanosoma b. rhodesiense* STIB 900 in 50 μL were added to each well, and the plate was incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Ten microliters of Alamar Blue (12.5 mg of resazurin dissolved in 100 mL of distilled water) was then added to each well, and incubation continued for a further 2–4 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA). Decrease of fluorescence (=inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC_{50} values were calculated. Cytotoxicity was assessed using the same assay and rat skeletal myoblasts (L-6 cells). The medium used for the L-6 cells was RPMI 1640 medium with 10% FBS and 2 mM L-glutamine.

Supporting Information Available: Copies of spectra and additional data are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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