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Edonamides, the first secondary metabolites from the recently described myxobacterium *Aggregicoccus edonensis*

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ABSTRACT

Two fatty acid styryl amides, edonamides A (**1**) and B (**2**), were isolated as the first secondary metabolites from the recently described myxobacterial genus and family *Aggregicoccus edonensis* strain MCy136. Their molecular structures were elucidated using extensive HRESIMS and NMR analyses. Since edonamides A (**1**) and B (**2**) did not show inhibitory activity against different bacteria, fungi, and cell lines a role as signaling molecules in the life cycle of myxobacteria seems possible.

Myxobacteria are fascinating soil dwelling bacteria with an extraordinary life style, unique in the bacterial kingdom. In times of nutritional deficiencies vegetative cells swarm together and form different species-specific fruiting bodies. Within these fruiting bodies, cells convert to dry-resistant myxospores, which are able to survive for many years. Not only for microbiologists but also for natural product chemists myxobacteria are of great interest. Their large genomes (9-10 million nucleotides) provide the best conditions for a rich secondary metabolism. More than 100 substance families, many of them with bioactivity, have been isolated from myxobacteria in the last 40 years. Antibiotics against Gram-negatives like cystobactamides,[1] against Gram-positives like myxopyronins,[2] potent fungicides like soraphens[3] and the cytostatic epothilones[4] and disorazol[5] are just a few examples of some of the best known myxobacterial secondary metabolites. Therefore myxobacteria play in the same league as actinobacteria and fungi as producers of bioactive secondary metabolites.[6] During our ongoing in-house screening for bioactive metabolites from myxo- and other gliding bacteria, especially new families, genera and even species turned out to be reliable sources for new types of secondary metabolites.[7-9] The genus *Aggregicoccus* belongs to the family Myxococcaceae, suborder *Cystobacterineae* and was up to date completely unexplored regarding the production of secondary metabolites.

Whereas species of the genus *Myxococcus* are well known as sources of numerous structurally diverse metabolites,[2,10-12] just a few or no substances are known from species of the genera *Corallococcus*[13] and *Pyxidicoccus* (all *Cystobacterineae*), for example. Strain MCy1366 is the type-strain of the recently described novel myxobacterial genus and species, *Aggregicoccus edonensis*[14] (DSM 27872[T]). In crude extracts of fermentation broths of MCy1366 two metabolites with *m/z* 260 and *m/z* 246 for $[M+H]^+$ could be detected by HPLC-UV-MS and did not produce any hits in our internal mass spectral database of myxobacterial compounds. Herein, we report the isolation and structure elucidation of the first two secondary metabolites from *Aggregicoccus edonensis*, the styrylamides **1** and **2**. Extensive HRESIMS and NMR analyses were used in their structure elucidation.

For metabolite production *Aggregicoccus edonensis* strain Ar1733 was inoculated in 70 liters of M7-2 medium supplemented with Amberlite XAD-16 resin and fermented at 37 °C for 19 days. The resin was recovered from the culture broth by sieving and a crude extract was eluted from the resin with methanol and acetone. The products were isolated from the crude extract using a sequence of solvent partitioning, silica gel flash chromatography and preparative RP-medium pressure liquid chromatography (MPLC).

The main compound obtained from *A. edonensis* was edonamide A (**1**)[15] (19 mg). HRESIMS of **1** indicated the molecular formula $C_{17}H_{25}NO$. The absorption maxima at 282 and 279 nm in the UV spectrum of **1** suggested an aromatic ring in the structure. All proton and carbon signals were visible in the 1D NMR spectra in deuterated acetone (Table 1). Those directly connected to each other were correlated by a $^1H,^{13}C$ HSQC-DEPT spectrum leaving the singlet at $\delta_H = 8.64$ ppm as NH signal. The $^1H,^1H$ COSY NMR spectrum confirmed a phenyl ring containing H-6 to H-10.

Table 1: NMR Data of edonamides A (**1**) and B (**2**) in $(CD_3)_2CO$ (^1H 700.4 MHz; ^{13}C 125.8 MHz).

Position	Edonamide A (**1**)		Edonamide B (**2**)	
	δ_C, mult	δ_H, mult (*J* in Hz)	δ_C, mult	δ_H, mult (*J* in Hz)
1	171.0, C		171.1, C	
2		9.22, br. d (8.4)		9.22, br d (8.8)
3	124.5, CH	7.53, d (14.6)	124.5, CH	7.53, dd (14.6, 10.5)
4	111.8, CH	6.15, d (14.8)	111.8, CH	6.15, d (14.6)
5	138.1, C		138.1, C	
8	7.13, tt (7.3, 1.2)		7.13, tt (7.3, 1.3)	
126.9, CH				

6/10	126.1, CH	7.33, dd (8.0, 1.3)	126.1, CH	7.33, dd (8.2, 1.1)
7/9	129.5, CH	7.27, t (7.5)	129.5, CH	7.27, t (7.7)
11	36.7, CH_2	2.29, t (7.4)	36.7, CH_2	2.28, t (7.4)
12	26.2, CH_2	1.65, quin (7.1)	26.2, CH_2	1.64, quin (7.4)
13	30.3, CH_2	1.32, m	29.9, CH_2	1.32, m
14	28.0, CH_2	1.32, m	29.9, CH_2	1.29, m
15	39.7, CH_2	1.18, m	32.6, CH_2	1.28, br s
16	28.7, CH	1.53, dquin (13.3, 6.7)	23.4, CH_2	1.29, m
17	23.0, CH_3	0.87, d (6.5)	14.4, CH_3	0.88, br t (7.1)
18	23.0, CH_3	0.87, d (6.5)		

^{13}C and ^{13}C DEPT NMR spectra indicated the presence of seventeen carbon atoms of which two were quaternary, C-3 to C-10 were aromatic or double bond methines, C-11 to C-15 were methylene-groups, C-16 was an aliphatic methine and C-17 and C-18 represented two methyl-groups. From their chemical shifts the quaternary carbons were assigned as a carbonyl group (C-1) and as the substituted phenyl carbon C-5. $^{1}H,^{13}C$ HMBC spectrum and coupling constants of H-6 to H-10 supported the presence of the phenyl ring, which could also be seen in the UV spectrum. Furthermore C-6 and C-10 showed $^{1}H,^{13}C$ HMBC correlations with H-4. The *E* configuration of the $\Delta^{3,4}$ double bond was confirmed by the typical coupling constant of 14.6 Hz while the expected coupling constant of a *Z* double bond would be around 10 Hz.

Selected relevant HMBC and ROESY correlations are shown in the resulting structure in Fig. 1.

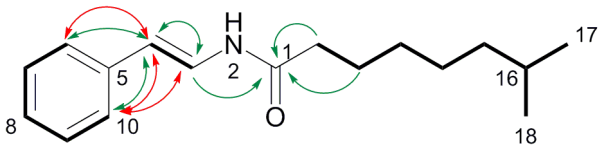

Figure 1: Selected 2D correlations for structure determination of **1** (gray zones $^{1}H,^{1}H$ COSY; green arrows $^{1}H,^{13}C$ HMBC; red arrows $^{1}H,^{1}H$ NOESY).

For edonamide B (**2**)[16] HRESIMS and isotopic pattern analysis of the molecular ion cluster $[M + H]^+$ suggested the molecular formula $C_{16}H_{23}NO$. ^{1}H NMR and 2D NMR correlations of **2** were almost identical to those of **1** (Table 1 and supporting information), with the exception of the signals corresponding to the isopropyl group. Instead, only one methyl group at C-16 was visible as a triplet at δ_H 0.88 ppm, which suggested C-16 being a methylene group.

1 and **2** were tested for antimicrobial and antifungal activity as well as for cytotoxicity against growing primary and cancer cell lines, namely the mouse fibroblast L929, breast cancer MCF-7, and epidermoid carcinoma A431. Neither **1** nor **2** showed any activity against the organisms tested and the IC_{50} values against the cell lines tested were above 30 µg/ml.

As edonamides A and B are new natural products we compared them with other known natural products. Natural products containing the styrylamide part (C-1 to C-10) of **1** and **2** are for example the very weak antifungal illukumbin[17] from *Glycosmis mauritiana* and the antibacterial xylaramides from the fungus *Xylaria longipes*.[18] Looking at the *iso*-branched fatty acid chain of **1** the same fatty acid amide is part of complex peptides such as the antibiotic MM55266 from an *Amycolatopsis* species.[19] Another very similar natural product , *N*-(7-methyl-octanoyl) 2-phenylethylamine from *Xenorhabdus doucetiae* did not show any biological activity, either. However, small changes in the fatty acid chain, for example removing a methyl-group, sufficed to get cometabolites with cytotoxic activity similar to **2**.[20]

Comparing the straight chain fatty-acid of **2** brought up quorum-sensing inhibitors like *N*-octanoyl-3-amino-2-cyclo-hexen-1-one from *Burkholderia glumae*,[21] siderophores such as corrugatin from *Pseudomonas corrugata*[22] and antibiotics like fragin from *Pseudomonas fragi*[23,24]. Also the fatty acid residue can be found in pigments like lemonnierin from *Pseudomonas lemonnieri*.[25]

Furthermore, myxobacteria produce a variety of different fatty acids with unknown biological function. However, Bode *et al.*[26] have shown *iso*-branched fatty acids play a role in the myxobacterial life cycle. A decrease in *iso*-branched fatty acid production resulted in a strongly delayed and reduced aggregation and sporulation. As **1** is an *iso*-branched fatty acid amide it might be possible that it has a function in myxobacterial growth and life cycle.

Up to date the biological role of edonamides A (**1**) and B (**2**) has not been found but will inspire further investigations in the future.

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Supplementary data

Supplementary data (experimental details; ^{1}H NMR, ^{13}C NMR, COSY, HSQC-DEPT, HMPC and ROESY spectra; UV spectra and IR spectra) associated with this article can be found.

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15 Edonamide A (**1**): $C_{17}H_{25}NO$, M = 259.19; HPLC : R*t* = 21.7 min; UV (MeOH) λ_{max} (log ε) 221 (3.96), 279 (4.2), 285 (4.2) nm; IR (ATR) ν_{max} 3297 (m), 3067 (w), 3028 (w), 2921 (m), 2864 (w), 1666 (m), 1642 (s), 1576 (w), 1522 (s), 1495 (m), 1463 (m) 1448 (m), 1415 (m), 1383 (m), 1365 (w), 1355 (w), 1318 (w), 1299 (w), 1277 (m), 1248 (s), 1238 (s), 1222 (m), 1192 (s), 1153 (w), 1075 (w), 1030 (w), 964 (m), 942 (s), 833 (w), 750 (s), 734 (m), 721 (s), 692 (s) cm^{-1}; ^{1}H and ^{13}C NMR see table 1; HRESIMS *m/z* 260.2010 $[M+H]^+$ (calcd for $C_{17}H_{26}NO^+$, 260.2009).

16 Edonamide B (**2**): $C_{16}H_{23}NO$, M = 245.18; HPLC : R*t* = 20.27 min; UV (MeOH) λ_{max} (log ε) 220 (4.09), 279 (4.32), 285 (4.32) nm; ^{1}H and ^{13}C NMR see table 1; HRESIMS *m/z* 246.1848 $[M+H]^+$ (calcd for $C_{16}H_{24}NO^+$, 246.1852).

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