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Cebulactams A1 and A2, new macrolactams isolated from *Saccharopolyspora cebuensis*, the first obligate marine strain of the genus *Saccharopolyspora*

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ABSTRACT

The new macrolactams cebulactams A1 and A2 were isolated from an extract of the first obligate marine strain of the genus *Saccharopolyspora*. Their constitutionally identical structures, each bearing a six-membered cyclic ether as part of the macrocycle, and their relative configurations were elucidated by MS methods and by 1D and 2D NMR techniques.

Terrestrial actinomycetes have proven to be a highly productive source of novel secondary metabolites.[1] However, due to the exhaustive scientific interest in these bacteria, the isolation of new chemical structures from actinomycetes has become a difficult task.[2] Diverse strategies to overcome this problem have been pursued, leading to the development of effective methods for dereplication and online structure elucidation by combining modern chromatographic and spectroscopic methods[3–8] as well as to improvements of culturing techniques for unusual microbes.[9,10] In recent years, marine actinomycetes have emerged as a particularly rich source of new secondary metabolites,[11,12] as demonstrated by the isolation of highly bioactive molecules derived from obligate marine bacteria, such as salinosporamide A (**1**)[13] and sporolide A (**2**).[14]

We have recently described the isolation and phylogenetic identification of a new species, *Saccharopolyspora cebuensis* type strain SPE 10-1, derived from the sponge *Haliclona* sp. collected by scuba diving offshore Cebu, Philippines.[15] This strain was found to require at least 25% artificial seawater for growth, showing that it is the first obligate marine strain of the genus *Saccharopolyspora*.[15] We herein describe the isolation and structural elucidation of two novel, constitutionally identical macrolactams, cebulactams A1 (**3**) and A2 (**4**), from this marine bacterium (Fig. 1).

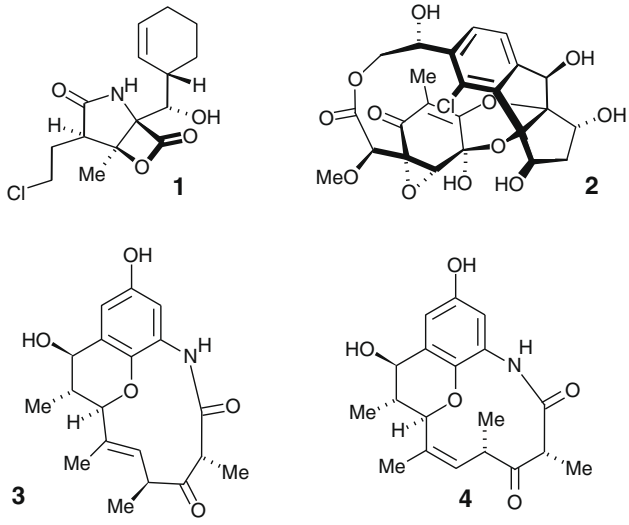

Figure 1. Structures of natural products isolated from obligate marine bacteria: salinosporamide A (**1**), sporolide A (**2**), and the herein described cebulactams A1 (**3**) and A2 (**4**).

HPLC–UV analysis of the extract of a culture filtrate of SPE 10-1[16] showed two prominent peaks in the polar region of the chromatogram. The UV spectra of these metabolites were almost identical, both exhibiting a strong maximum at 215 nm. In addition, the faster eluting metabolite, **3**, had an absorption maximum at

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291 nm, which was bathochromically shifted to 303 nm in the case of compound **4**. HPLC–ESI-MS analysis revealed the molecular masses of **3** and **4** to be identical (*m/z* 346.3 for $[M+H]^+$), suggesting the two compounds to be structurally highly similar to each other. These assumptions were further corroborated by HR-ESIMS (TOF) measurements, using the pure compounds as obtained by preparative HPLC,[17] which clearly showed an identical molecular formula of $C_{19}H_{23}NNaO_5$ (*m/z* 368.1478 for $[M+Na]^+$, calcd 368.1468) for **3** and **4**.

The ^1H NMR spectrum of **3** revealed the presence of 13 signals: besides three doublets of aliphatic methyl groups (δ_H 1.00, 1.19, and 1.21) and a singlet of a methyl group attached to a double bond (δ_H 1.86), the signals of five aliphatic CH functions (δ_H 1.82, 3.19, 3.43, 4.27, and 4.38), three hydrogens attached to sp^2-hybridized carbon atoms (δ_H 4.98, 6.65, and 7.02), and of a broad, hetero-atom bound proton (δ_H 7.88) were visible. In the ^{13}C NMR spectrum of **3**, all expected 19 signals were detectable. Besides the respective resonances for the proton-bearing carbon atoms, which were assigned by analysis of the HMQC NMR spectrum of **3** (Table 1), three sp^2 (δ_C 111.1, 114.0, and 124.7) and two oxygen-substituted sp^2 carbons (δ_C 142.7 and 154.4), as well as the resonances of a carboxy or carbamide function (δ_C 173.9) and a keto group (δ_C 207.1), were observed.

Analysis of COSY (↔) and HMBC (→) correlations facilitated the elucidation of three partial structures **3a**–**c** (Fig. 2). The assembly of these molecular parts to give **3d** was possible using HMBC interactions from H-3 to C-4 and Me-4, from Me-4 to C-3 and C-7, from H-5 to C-3 and C-7, from H-6 to Me-6 and C-7, and from H-8 to Me-8, C-7, and C-9.

The aromatic protons H-13 (δ_H 7.02) and H-15 (δ_H 6.65) showed a coupling constant of 2.8 Hz, suggesting these two atoms being located in *meta*-positions to each other. HMBC interactions from H-13 to C-11, C-14, and C-15, and from H-15 to C-10, C-11, C-13, and C-14 led to partial structure **3e** (Fig. 3). Further HMBC correlations from H-13 to C-1 in **3d** and from H-1 to C-11, C-12, and C-13 in **3e** allowed the connection of these two molecular portions. A strong HMBC cross peak between H-3 and C-11 as well as the chemical shifts of C-3 (δ_C 87.4) and C-11 (δ_C 142.7) clearly evidenced an additional connection of **3d** and **3e** via a cyclic ether. Comparison of the calculated molecular mass of the resulting working structure with the experimentally observed MS data for **3** indicated that a nitrogen atom and three protons were still missing. Partial structure **3f** thus bears hydroxy groups, both at C-1 and

Table 1
NMR spectroscopic data of cebulactam A1 (**3**) in acetone-d_6 (^1H: 400 MHz; ^{13}C: 100 MHz)

Position	δ_C	δ_H, mult	COSY (*J* in Hz)	HMBC	NOESY
1	70.9	4.38 d	2 (10.1)	2, Me-2, 3, 11, 12, 13	Me-2
2	42.4	1.82 m	Me-2, 1, 3	1, Me-2, 3, 12	5
Me-2	16.7	1.19 d	2 (6.6)	1, 2, 3	1, 3
3	87.4	4.27 d	2 (8.5)	2, Me-2, 4, Me-4, 5, 11	Me-2
4	140.5				
Me-4	19.3	1.86 s	5	3, 4, 5, Me-6, 7	6
5	124.7	4.98 d	Me-4, 6 (9.8)	3, Me-4, 6, 7	2, Me-6, 8
6	46.2	3.19 m	Me-6, 5	4, 5, Me-6, 7	Me-4
Me-6	17.8	1.00 d	6 (6.3)	5, 6, 7	5, 8
7	207.1				
8	48.2	3.43 q	Me-8 (6.8)	7, Me-8, 9	5, Me-6
Me-8	16.6	1.21 d	8 (6.8)	7, 8, 9	
9	173.9				
10	131.0				
11	142.7				
12	140.9				
13	111.1	7.02 d	(2.8)	1, 11, 14, 15	
14	154.4				
15	114.0	6.65 d	(2.8)	10, 11, 13, 14	
NH		7.88 br			

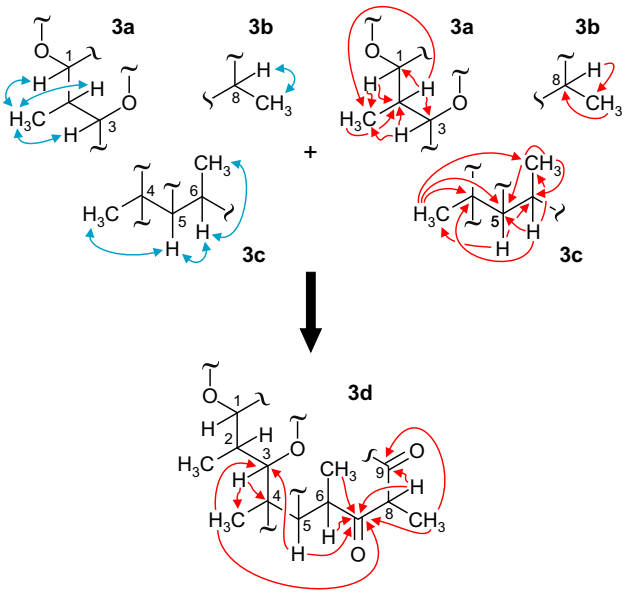

Figure 2. Assembly of partial structures **3a**–**c** to give **3d** using COSY (↔) and HMBC (⟶) interactions.

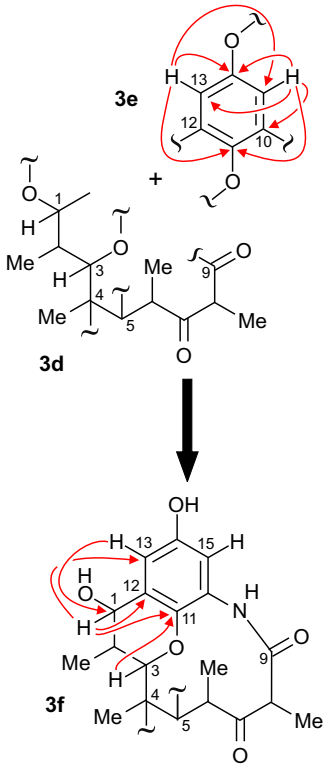

Figure 3. Connection of the molecular portions **3d** and **3e** using HMBC correlations (⟶) to give the extended partial structure **3f**.

at C-14, and a nitrogen function at C-10 forming an amide bond with the carbonyl C-9 (Fig. 3).

The configuration at the double bond between C-4 and C-5 and the relative configurations at the stereocenters at C-1, C-2, C-3, C-6, and C-8 in **3** were investigated by NOESY-NMR spectroscopy. Me-4 and H-5 did not exhibit any cross coupling, thus indicating the geometry of the respective double bond to be *trans*. Strong couplings between H-1↔Me-2↔H-3 clearly evidenced their spatial

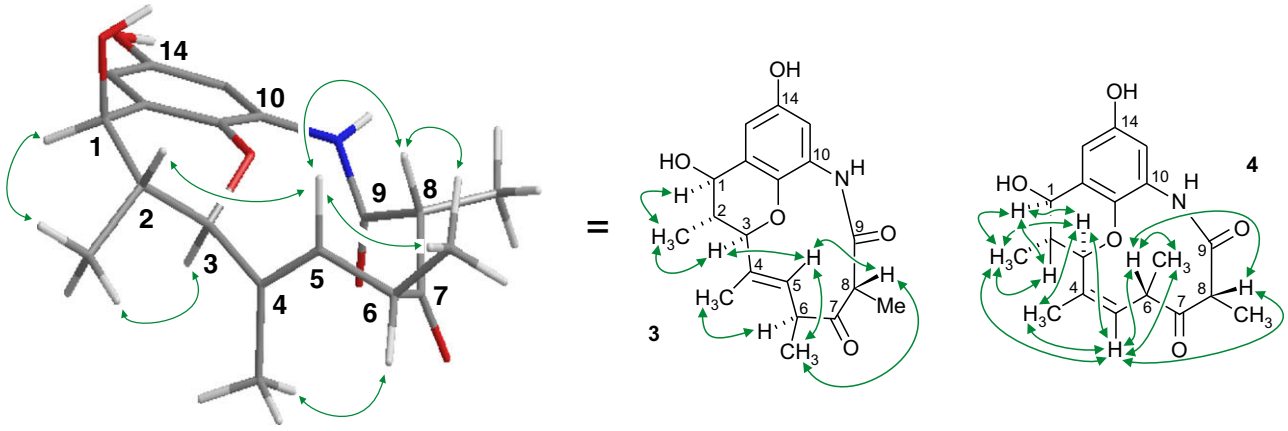

Figure 4. Double bond and relative configurations in cebulactam A1 (**3**) and A2 (**4**) as elucidated by NOESY interactions (↔).

arrangement on the same side of the macrocycle. Correlations between H-2↔H-5 and H-5↔Me-6↔H-8↔H-5 on the one hand, and Me-4↔H-6 on the other defined the relative position of these groups in the molecule, leading to the overall relative stereostructure **3** for cebulactam A1 (Fig. 4). The observed large coupling constants between H-1 and H-2 (10.1 Hz), H-2 and H-3 (8.5 Hz), and between H-5 and H-6 (9.8 Hz) fully supported this conclusion.

The NMR spectra of the slower-eluting peak **4** (Table 2) were closely related to those recorded for cebulactam A1 (**3**), the decisive difference between these compounds being found in the NOESY data. This time, the substituents located at the double bond, Me-4 and H-5, exhibited a strong cross peak, evidencing the double bond to be cis-configured in cebulactam A2 (**4**, Fig. 4), all other details, including the full relative configuration, being identical for **4** and **3**.[18]

Cebulactams A1 (**3**) and A2 (**4**) thus represent constitutionally identical marine derived macrolactams with a unique cyclic ether connecting the aromatic ring system to the highly functionalized, polyketide-derived carbon chain. The only structurally related secondary metabolites known so far are compounds isolated from *Pseudonocardia* Q-1047.[19–21] These natural products, however, are all lacking the cyclic ether structural motif. Instead, they bear either a *para* dihydroxylated aromatic ring system, or the respective oxidized benzoquinone, and additionally are in part further functionalized by O-glycosidation at the C-1 hydroxy function. Interestingly, the compounds isolated from Q-1047 display remarkable antioxidant activities.[19–21] The evaluation of the biological potential of cebulactams A1 (**3**) and A2 (**4**) is in progress.

Table 2
NMR spectroscopic data of cebulactam A2 (**4**) in acetone-d_6 (^{1}H: 400 MHz; ^{13}C: 100 MHz)

Position	δ_C	δ_H, mult	COSY (J in Hz)	HMBC	NOESY
1	70.8	4.36 d	2 (10.1)	2, Me-2, 3, 11, 12, 13	2, Me-2, 3
2	46.2	1.51 m	Me-2, 1, 3	1, Me-2, 3, 12	1, Me-2
Me-2	15.0	1.15 d	2 (6.6)	1, 2, 3	1, Me-2, Me-4, 5
3	86.2	4.24 d	2 (10.0)	2, Me-2, 4, Me-4, 5, 11	Me-2
4	145.4				
Me-4	19.8	2.02 s	5	3, 4, 5	3, 5
5	125.1	5.07 d	Me-4, 6 (10.5)	3, Me-4, 6, 7	Me-2, 3, Me-4, 6, Me-6, 8
6	48.3	3.34 m	Me-6, 5	4, 5, Me-6, 7	5, Me-6, 8
Me-6	16.9	0.99 d	6 (6.8)	5, 6, 7	5, 6
7	205.2				
8	55.6	3.97 q	Me-8 (6.7)	7, Me-8, 9	5,6
Me-8	15.1	1.27 d	8 (6.7)	7, 8, 9	
9	173.3				
10	130.4				
11	139.5				
12	138.1				
13	107.3	6.76 d	(2.8)	1, 11, 14, 15	
14	153.4				
15	110.9	6.83 d	(2.8)	10, 11, 13, 14	
NH		8.61 br			

Acknowledgments

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16. Cultivation conditions: 100 µl of a glycerol stock of SPE 10-1 were inoculated in 3 × 100 ml of ISP 2 medium (per l of artificial seawater: yeast extract 4 g, malt extract 10 g, dextrose 4 g), incubated with shaking at 180 rpm at 30 °C for 5 d. An aliquot of 50 ml of the inoculum was then transferred to 6 × 750 ml of fresh ISP 2 medium, and the cultures were incubated under the same conditions for further 7 d. After incubation, methanol was added to each culture (1:2) with shaking at 20 °C overnight. The resulting mixtures were filtered and extraction with half-volume of ethyl acetate was done twice. The ethyl acetate layers were separated and dried by rotary evaporation.
17. The isolation of compounds **3** and **4** was carried out by preparative HPLC (Merck Chromolith SemiPrep RP18e 10 × 100 mm) using H_2O + 0.05% TFA (A)

and MeCN + 0.05% TFA as the solvents, and the following gradient: flow 10 ml/min; 0 min 75% B, 10 min 75% B yielding 5.1 mg of **3** (R_t = 2.5 min) and 4.1 mg of **4** (R_t = 4.3 min).

18. The occurrence of the *E* and the *Z* isomer of the C-4/C-5 double bond in cebulactams A1 (**3**) and A2 (**4**), respectively, might hint at a possible interconversion of these compounds, either induced by unsuitable isolation conditions or simply catalyzed by light. This, however, seems to be excluded, because the pure isomers, **3** and **4**, are configuratively stable under the isolation conditions and, hence, their ratio did not change significantly when using different workup protocols.
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