Cyaneodimycin, a Bioactive Compound Isolated from the Culture of Streptomyces cyaneofuscatus Associated with Lichina confinis
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**Methacrylate Natural Products**

**Cyaneodimycin, a Bioactive Compound Isolated from the Culture of Streptomyces cyaneofuscatus Associated with Lichina confinis**


Abstract: Two new compounds, cyaneodimycin (1) and cyaneomycin (2) bearing a rare methacrylate residue, were isolated from cultures of the bacterium *Streptomyces cyaneofuscatus* associated with the marine lichen *Lichina confinis*. These astonishing structures may be derived from a pyruvate biosynthetic pathway. Cyaneodimycin (1) exhibited antiproliferative properties against B16 (IC₅₀ = 27 ± 4 μM), HaCaT (IC₅₀ = 47 ± 11 μM) and Jurkat cell lines (IC₅₀ = 18.5 ± 0.5 μM). Six known compounds 3–8 with diversified structures and interesting biological properties (diketopiperazines, actinomycin, dibenzoferane or indole derivatives) were isolated jointly with compounds 1 and 2. Among them, usnic acid (8), a common lichen compound was isolated for the first time from bacteria.

**Introduction**

Many drugs originate from microorganisms.[1] Additionally, the study of microorganisms from unexplored habitats often results in new chemistries, therapeutic leads and insights into novel bioactive principles, especially if those habitats foster symbiotic relationships among organisms.[2] Among underexplored natural product sources, lichens are unique and are classically described as a symbiotic association between a photobiont (green algae and/or cyanobacteria) and a mycobiont. Long-lived lichen symbioses provide an ecological niche for highly diverse additional microorganisms observed by culture-dependent[3] and -independent approaches.[4–10] Examinations of natural-product profiles of these underexploited bacteria associated with lichens have already started, and have revealed new compounds with cytotoxicity and/or antimicrobial activities,[11] such as angucycline and butenolid,[12] coumabiocins A–F,[13] cladonamides A–G,[14] uncialamycin[15] and, more recently, uncianphenol.[16] As part of our program focused on the discovery of new natural products from lichen-associated bacteria, we selected an interesting bacterial strain (MOLA 1488) associated with *Lichina confinis* for further study; the EtOAc extract of MOLA 1488 fermentation was found to display significant cytotoxicity against B16 cell lines (IC₅₀ = 0.33 ± 0.2 μg/mL). Fermentation of this strain led to the isolation of two new compounds (both methacrylate derivatives) and six known compounds belonging to various structural families (diketopiperazines, indole, phenoxazin or dibenzoferane derivatives). Some of the isolated compounds demonstrated interesting cytotoxic activities. We describe herein the isolation, structural identification and biological properties of cyaneodimycin (1), a new methacrylate derivative, jointly with the structural elucidation of cyaneomyacin (2).

**Results and Discussion**

The producing bacterial strain MOLA1488 was isolated from a marine lichen, *Lichina confinis* collected on the Brittany coast (Erquy, France). Characterization by 16S rRNA gene sequencing showed the strain to be related at 100 % to *Streptomyces cyaneofuscatus* JCM 4364 after comparisons using the EzTaxon database server.[17] A relatively large-scale (2.7 L) shake fermentation was carried out using modified LB medium over the course of 12 d, to obtain quantities of material sufficient to support full chemical and biological analysis of the metabolites. The excreted metabolites were collected using sterile XAD-7-HP resin, and the solid residue (constituted of resin and bacterial cells) was first extracted three times with acetone/MeOH (50:50, v/v). Concentration of the acetone/MeOH extracts in vacuo...
gave a brownish residue that was partitioned between EtOAc and H₂O. The EtOAc extract, corresponding to the crude material, was used for all experiments (80 mg). A dereplication approach using GNPS (Global Natural Products Social molecular Networking platform at gnps.ucsd.edu, Wang et al.¹¹⁸) based on MS² fragmentation spectra similarities permitted highlighting of a cluster of diketopiperazines including cyclo-[(L-Leu,L-Pro), cyclo-(L-Pro,L-Val), cyclo-(L-Phe,L-Pro), cyclo-(Leu,Leu), cyclo-(Leu,Phe), cyclo-(Val,Leu), cyclo-(Val,Phe)] (see Supporting Information, Figure S1). Notably, some biological activities had already been reported for this chemical family (antibacterial, antifungal, antifouling, cytotoxic properties among others).¹⁹–²¹

Flash chromatography and semipreparative HPLC runs were performed on the EtOAc extract (80 mg, viscous aspect) and led to the isolation of two new compounds 1 (1.5 mg) and 2 (0.2 mg).

Cyaneodimycin (1) was isolated as a yellow residue with HRESIMS data of an [M + Na]⁺ ion at m/z = 379.17327, appropriate for a molecular formula of C₁₈H₂₈O₇. The ¹H NMR, JMOD and 2D (COSY, TOCSY, HSQC and HMBC) data were acquired in CD₂Cl₂. These data (¹H NMR and JMOD) revealed the presence of the following groups: carbonyl carbon atoms (δ_C = 167.3 and 176.1 ppm), a methylene group (δ_C = 125.6 ppm and δ_H = 5.54 and 6.04 ppm) and a quaternary carbon atom (δ_C = 136.7 ppm). Signals of three OCH₂ functions (δ_C = 73.4 and 71.5 ppm; δ_H = 3.46–3.56 and 3.36–3.41 ppm; δ_H = 4.06 ppm), four methyl groups (δ_C = 18.4, 13.6 and 7.6 ppm; δ_H = 1.90, 1.12 and 0.87 ppm), one CH group (δ_C = 39.9 ppm; δ_H = 2.69–2.73 ppm) and one methylene group (δ_C = 23.6 ppm; δ_H = 1.48 ppm) were also observed in the aliphatic region. On the other hand, ¹H NMR spectroscopic data of 1 revealed the presence of two methylene protons (δ_H = 5.54 and 6.04 ppm) as each of the signals integrated for two protons. Similar observations were shown for signals at δ_H = 1.90 and 4.06 ppm integrating for four and six protons, respectively, and suggesting the presence of two identical methyl groups (C-13 and C-15, δ_C = 18.4 ppm) and two OCH₂ functions (C-3 and C-10, δ_C = 64.9 ppm). ¹H-¹H COSY experiments highlighted correlations between protons H-14 (δ_H = 5.54 and 6.04 ppm) and H-13 (δ_H = 1.90 ppm) and, on the other hand, between protons H-16 (δ_H = 5.54 and 6.04 ppm) and H-15 (δ_H = 1.90 ppm). Additional ¹H-¹³C HMBC analyses showed connection between H-14 (δ_H = 5.54 and 6.04 ppm) and carbon atoms C-12 (δ_C = 136.7 ppm) and C-11 (δ_C = 167.3 ppm), and H-16 (δ_H = 5.54 and 6.04 ppm) and carbon atoms C-2 (δ_C = 136.7 ppm) and C-1 (δ_C = 167.3 ppm). These observations are consistent with the presence of two identical α,β-unsaturated carbonyl groups in the structure of 1. Moreover, a 3-hydroxy-2-methylpropanoate fragment was identified on the basis of COSY correlations between H-8 (δ_H = 3.46–3.56 ppm), H-7 (δ_H = 2.69–2.73 ppm) and H-9 (δ_H = 1.12 ppm), and HMBC correlations between H-7 (δ_H = 2.69–2.73 ppm) and C-6 (δ_C = 176.1 ppm). At least one ethylene moiety was assigned using COSY correlations between H-17 (δ_H = 1.48 ppm) and H18 (δ_H = 0.87 ppm). Other HMBC correlations from H-3 and H-10 to C-4, C-5 and C-17 allowed the connectivity between the three identified fragments and led to unambiguous assignment of the structure of 1 (Figure 1; Supporting Information, Figures S2–S4).

![Figure 1. Key COSY (black lines) and HMBC (arrows H to C) correlations for cyaneodimycin (1).](image-url)

Similar ¹H NMR assignments were observed for compound 2 with two major differences: (i) a further CH₂OH group (signal at δ_H = 3.57–3.67 ppm) and (ii) a methylene group (signal at δ_H = 5.62 and 6.14 ppm) integrating only for two protons relative to cyaneodimycin (1) (Table 1; Supporting Information, Figures S2 and S7). By analogy with the structure of 1, we propose the chemical structure for cyaneomycin (2) shown in Figure 2.

Interestingly, HRESIMS analysis of compound 2 led to an [M + H]⁺ ion at m/z = 203.0765, consistent with a formula of C₁₃H₁₉O₄. The difference from the molecular formula of 1 corresponds to the loss of two C₆H₅O₂ moieties, which could be

### Table 1. NMR spectroscopic data for cyaneodimycin 1 and cyaneomycin 2 at 500 MHz.

<table>
<thead>
<tr>
<th>Cyaneodimycin (1) (CDCl₃)</th>
<th>Cyaneomycin (2) (CDCl₃)</th>
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</thead>
<tbody>
<tr>
<td><strong>Position</strong></td>
<td><strong>Carbon type</strong></td>
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<tr>
<td>1/12</td>
<td>C (quaternary)</td>
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<tr>
<td>2/11</td>
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<td>CH₂</td>
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<tr>
<td>16</td>
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[a] HMBC correlations are from proton(s) to the indicated carbon. [b] n.d.: not determined due to masking by water signal.

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explained by a hydrolysis process during the deuterium exchange (after the NMR analysis and before or during the MS/MS analysis). All assignments in the 1H NMR data were nevertheless observed for cyaneomycin (2) (Table 1 and Supporting Information S7). Fragmentation mass spectrometry analysis (MS/MS) confirmed the presence of these new compounds and inferred the proposed structure. Indeed, several fragments were observed corresponding either to a loss of one, two or three C₇H₆O₂, H₂O groups or more complex structures (see Supporting Information S5–S6 and S8–S9). Nonetheless, it is not possible to exclude the possibility that 2 may also be an artefact formed by hydrolysis of 1 during the extraction process of S. cyaneofuscatus. The absolute configuration of C7 in compound 1 was determined to be (R) based on its optical rotation [α]D₂₀ = −9 (c = 0.1, CHCl₃) relative to that of commercially acquired (–)-3-hydroxy-2-methylpropionate with [α]D₂₀ = −14 (c = 0.1, CHCl₃). The absolute configurations of C4 and C7 in compound 2 remain unknown. Attempts at crystallization failed, and the use of electronic circular dichroism methods did not render any exploitable CD signatures. These failures could be explained by the high degree of flexibility of both molecules and by the distant positioning of the chromophore group from the asymmetric carbon atom in 2. Unfortunately, the small amount of compound 2 that was obtained did not allow derivatization steps, which may be a good alternative to reduce those structural constraints. Cyaneodimycin (1) and cyaneomycin (2) represent rare examples of acrylate compounds from bacterial fermentation. To the best of our knowledge, 1 and 2 join only three other related natural products: cyrmenins A, B1 and B2, which may be a good alternative to reduce those structural constraints. Cyaneodimycin (1) and cyaneomycin (2) represent rare examples of acrylate compounds from bacterial fermentation.

Figure 2. Chemical structure of cyaneomycin 2.

Figure 3. Proposed metabolic synthesis of methacryloylCoA,[24]

whose structural compositions are: cyclo-(Phe,Pro) (4) (1.2 mg), cyclo-(L-Leu, L- Pro) (5) (3.6 mg), N-acetyl-β-oxotryptamine (6) (2.2 mg) and N-methyladactinomycin (7) (1 mg). The isolation of 4 and 5 confirmed the validity of our dereplication approach. The molecular formula of 7 was determined to be C₉₂H₈₈N₁₂O₁₆ (with 26 points of unsaturation) based on HRESIMS and NMR analyses. This compound also showed a maximum absorbance at λmax = 441 nm (in MeOH). All data revealed similarities with actinomycin D with the presence of one additional N-methyl group (δH = 2.81 ppm; δC = 54.80 ppm) (see Supporting Information S14 and S15). Compound 7 corresponds to the N-methyladactinomycin on the basis of comparisons with available data.[31] However, the chemical structure given in a patent[31] presents two structural errors but with correct NMR assignments. Consequently, we revised herein the chemical structure of N-methyladactinomycin (7) (Figure 4, see Supporting Information S14).

Figure 4. Known compounds 3–8 isolated from Streptomyces cyaneofuscatus MOLA1488: 3: (3-hydroxyacetyl)indole; 4: cyclo-(L-Phe, Pro); 5: cyclo-(L-Leu, L-Pro); 6: N-acetyl-β-oxotryptamine; 7: N-methyladactinomycin ; 8: usnic acid.

Among the known compounds, usnic acid (8) (1 mg), a common cytotoxic secondary metabolite in lichens[30,32–35] was isolated and identified for the first time from bacteria.[36] Even though usnic acid is a very widespread lichen compound and is responsible for the green to greenish-yellow color of many
lichens, this lichen compound has never been reported for the species L. confinis from which the present S. cyaneofuscatus producer was isolated. The molecular formula of C_{18}H_{16}O_{7} obtained by HRESIMS and additional MS/MS and NMR spectroscopic data confirmed, on the basis of comparisons with available data, the structural composition of metabolite B and its presence in the fermentation of S. cyaneofuscatus (see Supporting Information S16–S18). None of the compounds presented herein (1–8) were reported before. In fact, only four bioactive compounds such as daunomycin, cosmomyein, galtomycin B and maltophillin were putatively detected (exclusively by HPLC-UV/Vis) from S. cyaneofuscatus (strain M27) isolated from an unknown lichen.

The MOLA1488 crude extract showed cytotoxic activities in cell viability assays (MTT assay) against murine melanoma cells (B16 cell lines) with an IC_{50} of 0.33 ± 0.2 μg/mL but also against HaCaT cell lines (normal cell lines) with an IC_{50} of 0.25 ± 0.1 μg/mL. Pure cyaneodimycin (1) was also tested for cytotoxic properties against the same cell lines and showed an IC_{50} of 27 ± 4 μM and 47 ± 11 μM against B16 and HaCaT cell lines, respectively. By comparison, doxorubicin, used as positive control, exhibited an IC_{50} of 0.008 ± 0.001 μM against B16 and an IC_{50} of 0.15 ± 0.08 μM against HaCaT cells. In addition, the activity of 1 was evaluated using leukemic cell lines (Jurkat cell lines) and showed an IC_{50} of 18.5 ± 0.5 μM after 72 h of incubation. The same compound was found to be less active (IC_{50} > 20 μM) after a 16 h incubation with Jurkat cells. The biological properties of cyaneomycin (2) were not determined due to its low availability. It was also not surprising that actinomycin showed potent cytotoxic activities (IC_{50} ≈ 0.05 μM) as a result of the presence of compound 1. Indeed, actinomycin compounds are well-known antitumor agents by virtue of their ability to bind DNA and inhibit RNA synthesis.[39] As a result, the presence of compound 7 explains the potent cytotoxic activities of the extract.

**Conclusions**

We have successfully isolated two new compounds: cyaneodimycin (1) and cyaneomycin (2) from Streptomyces cyaneofuscatus associated with Lichina confinis, and compound 1 showed interesting cytotoxic properties. Six previously known compounds were also isolated and reported for the first time from this bacterial species. Moreover, we have unexpectedly isolated a common lichen compound [usnic acid (8)] from a bacterial strain. This important observation highlights the possibility of horizontal gene transfer between partners in lichen symbioses. Phylogenetic analysis of polyketide synthase genes have already indicated such horizontal gene transfer events in fungi for the salicylic acid biosynthesis.[40,41] In the present case, S. cyaneofuscatus might also be associated with other lichens, in particular those known for usnic acid production, unless Lichina is capable of producing usnic acid. However, this capability has not been demonstrated in previously studied individuals. Further experiments and the full genome sequencing of S. cyaneofuscatus MOLA1488 are ongoing and will allow us to elucidate the evolutionary background of usnic acid biosynthesis in this bacterium as well as in lichens. From such efforts we will develop a better understanding of the biosynthetic production of methacrylate compounds in this bacterium.

**Experimental Section**

**General Experimental Procedures:** All commercial reagents were purchased from Carlo Erba Reactifs and/or from Sigma Aldrich (Val-de-Reuil, France and St Quentin Fallavier, France). For chromatographic analysis, HPLC and LC/MS grade water was obtained from an EasyPure (Barnstead, USA) water purification system. Deuterated solvents were purchased from Euriso-top (Gif-sur-Yvette, France). All spectra were recorded with a Bruker DMAX 300 spectrometer [300 MHz (1H) and 75 MHz (13C) and Bruker 500 cryo-spectrometer (500 MHz (1H) and 125 MHz (13C)) using adequate deuterium solvents. Chemical-shift values were referenced to residual solvent signals for CDC13 (δ_H = 7.21/77.16 ppm) and CD2Cl2 (δ_H = 3.52/33.84 ppm). HSOIC, HMBC, COSY or TOCSY data were recorded using a Bruker DMAX 500 cryo-spectrometer instrument. NMR spectroscopic data were processed using the MestReNova version 1.3 software. Optical rotations were measured using a Perkin–Elmer Model 341 polarimeter at 20 °C using a thermostable optical glass cell (1 dm path length and c in g/100 mL). HRMS measurements for exact mass determination were performed with a MICROMASS ZabspecTOF spectrometer for electrospray ionization at the CRMPO (Centre Régional de Mesures Physiques de l’Ouest), University of Rennes 1.

**Microorganism:** Streptomyces cyaneofuscatus MOLA1488 (gene bank accession number: KM273905.1) was isolated from the lichen Lichina confinis collected in Erquy (Northwest of Rennes, France, 48°37′45″ N, 02°28′30″ W) in April 2012.[38] To identify the strain, its 16S rRNA gene was sequenced using dyeexoeX termination Sanger sequencing as described previously.[39] Comparisons with sequences in the EzTaxon strain database[17] revealed that the closest phylogenetic neighbor of the strain was Streptomyces cyaneofuscatus ATY999770, at 100 % sequence identity. The bacteria were stored after growth in Luria-Bertani broth medium (LB) [5 g yeast extract (Sigma–Aldrich, St Louis, MO), 10 g malt extract (Sigma–Aldrich, St Louis, MO) and 5 g NaCl (Sigma–Aldrich, St Louis, MO) for 1 L] with 50 % (v/v) glycerol or 5 % (v/v) DMSO at –80 °C (Banyuls/Mer collection, reference: MOLA1488).

**Fermentation of Streptomyces cyaneofuscatus MOLA1488:** MOLA1488 was cultivated in 50 mL test tubes containing 30 mL of LB medium [5 g yeast extract (Sigma–Aldrich, St Louis, MO), 10 g malt extract (Sigma–Aldrich, St Louis, MO) and 5 g NaCl (Sigma–Aldrich, St Louis, MO) for 1 L] with 50 % (v/v) glycerol or 5 % (v/v) DMSO at ~80 °C (Banyuls/Mer collection, reference: MOLA1488).

**Extraction and Isolation:** The solid residue of 2.7 L of fermentation culture was extracted three successive times with acetone/MeOH (50:50, v/v) (36.23 g). Acetone/MeOH extracts were dried in vacuo and three further successive extractions of the dried extract with
EtoAc/H2O (3:1, v/v) were realized. The organic phase (EtOAc extract) was collected and filtered using a Büchner funnel. The residual H2O in this phase was removed by adding anhydrous sodium sulfate powder (5 g), and the organic phase was dried under vacuum to yield ca. 80 mg of crude extract. The latter was fractionated using various methods [SPOT flash liquid chromatography (Armen Instrument®), semipreparative HPLC and preparative TLC]. For flash liquid chromatography, the stationary phase was a silica column pre-packed normal phase SiO2 (F3SHP-12007-0025, 25 g, Biotage), and the mobile phase was a gradient: CH2Cl2 (A)/EtOAc (B) (100:0 to 0:100 in 75 min). The following gradient was applied at a flow rate of 10 ml/min: initial, 100 % A; 0–10 min, 100 % A linear; 10–40 min, 50 % A linear; 40–45 min, 50 % A linear; 45–75 min, 0 % A linear, followed by washing by the column with 100 % MeOH in 30 min. 105 fractions of 10 ml each were collected. Fractions 15–18 (f1) (68.5 mg viscous) and 19–22 (f2) (4.5 mg) were purified by semipreparative TLC (10–12 μm, Kieselgel 60 F254, Merck 5554) using an automatic TLC sampler III, Camag®. Two solvent systems were applied: CH2Cl2/EtOAc/MeOH (15:15:3) for compound 3 and EtOAc (100 %) for compounds 4–7. Finally, five compounds were obtained in this manner.

3-(Hydroxacetyl)indole (3): Pale yellow powder. Rf = 0.36 in CH2Cl2/EtOAc/MeOH (15:15:3).1H NMR and 13C NMR data (CDCl3, 125 MHz) as described in the literature.[26] HRESIMS: m/z = 198.052558 [M + Na]+ (calcd. for C12H12N2O2Na 198.051453).

Cyclo-(Phe,Pro) (4): Yellow powder. Rf = 0.50 in EtOAc (100 %).1H NMR and 13C NMR data (CDCl3, 125 MHz) as described in the literature.[26] HRESIMS: m/z = 233.124952 [M + Na]+ (calcd. for C14H16N2O2Na 233.124952).

N-Acetyl-ß-oxyoctramine (6): Yellow powder. Rf = 0.75 in EtOAc (100 %).1H NMR and 13C NMR data (CDCl3, 125 MHz) as described in the literature.[25] HRESIMS: m/z = 239.078005 [M + Na]+ (calcd. for C12H19NO3Na 239.078002).

N-Methylactinomycin (7): Yellow powder. Rf = 0.59 in EtOAc (100 %). λmax(MeOH) = 441 nm.1H NMR data (CDCl3, 300 MHz) and 13C NMR data (CDCl3, 125 MHz) available in Table S1 and comparable to literature values.[31] HRESIMS: m/z = 258.3300 [M + H]+ (calcd. for C17H21NO2+ 258.3300). Separation and purification by semipreparative HPLC was also performed from the EtOAc extract (compounds 1–2) or from F1 (compound 8) using as first system (S1) a Kromasil C18, 100A column (5 μm, 250 × 10 mm). A gradient system was applied: A (water) and B (acetone) for 25 min at a flow rate of 2.5 mL/min in the HPLC system: initial, 100 % A; 0–5 min, 100 % A linear; 5–10 min, 90 % A linear; 10–15 min, 75 % A linear; 15–17 min, 75 % A linear; 17–25 min, 50 % A linear; 25–27 min, 50 % A linear; 27–30 min, 0 % A linear; followed by washing and reconditioning of the column. For compound 2 a semipreparative HPLC was performed using as second system (S2) a PreAlv C18 (5 μm, 250 × 10 mm) column with the following gradient: A (water) and B (acetone) performed at a flow rate of 2.5 mL/min in the HPLC system: initial, 100 % A; 0–5 min, 100 % A linear; 5–35 min, 0 % A linear; 35–45 min, 0 % A linear. Three compounds (1–2 and 8) were isolated.

Cyanodermicin (1): Yellow powder. [α]D20 = −9 (c = 0.1, CHCl3), tR = 27.50 min in acetone/H2O gradient (S1).1H NMR, 13C NMR and 2D NMR data (CDCl3, 500 MHz) are available in Table 1. HRESIMS: m/z = 379.171590 [M + Na]+ (calcd. for C18H28O7Na 379.171628).

Cyanomycin (2): Yellow powder. tR = 22.96 min in acetone/H2O gradient (S2).1H NMR data (CDCl3, 500 MHz) are available in Table 1. HRESIMS of the cyanomycin hydrolyzed: m/z = 203.1277 [M + H]+ (calcd. for C10H15O2Na 203.12833).

Usnic Acid (8): Pale yellow powder. tR = 9.55 min in acetone/H2O gradient (S1).1H NMR data comparable to literature values.[42] HRESIMS: m/z = 343.0824 [M – H]– (calcd. for C18H15O7Na 343.08236).

Mass Spectrometry Analysis: Mass spectrometry analysis was carried out using an LC-ESI and ESI-MS mass spectrometers as already described.[35,36] A PreAlv C18 column (5 μm, 250 × 4.6 mm, GRACE®) kept at 30 °C was used. For HPLC a gradient system was applied: A (0.1 % formic acid in water) and B (0.1 % formic acid in acetonitrile). The following gradient was applied at a flow rate of 1 mL/min in the HPLC system: initial, 99 % A; 0.1–5 min, 90 % A linear; 5–7 min, 90 % A linear; 7–15 min, 75 % A linear; 15–17 min, 75 % A linear; 17–25 min, 50 % A linear; 25–27 min, 50 % A linear; 27–40 min, 0 % A linear; followed by washing and reconditioning of the column. A split to 0.2 mL/min was applied before the mass spectrometry system. 20 μL were injected. The MS© spectra were recorded during the HPLC run using the following conditions: MS/MS analysis with starting collision-induced dissociation energy of 35 eV. The Xcalibur 1.0 software was used for data analyses. Mass spectrometry data were analyzed (molecular networking techniques, dereplication workflow, etc.) with the GNPS Platform (GNPS at http://gnps.ucsd.edu).[18]

Biological Assays: Cytotoxic properties of extracts and pure compounds were determined with a standard tetrazolium-based assay.[46] For the total EtOAc organic extract and cyanodermicin (1), B16 cells were seeded at 15000 cells/mL and HaCaT cells were seeded at 17000 cells/mL in RPM1640 medium with 5 % fetal calf serum (FCS) at day 0 in the appropriate well plates. Incubation was performed at 37 °C in an atmosphere of 5 % CO2. After 48 h of incubation, cell growth and viability were measured at 540 nm, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The cytotoxic activity of cyanodermicin (1) was determined against Jurkat cells seeded at 40000 cells/mL in RPMI media after 16 h (cytotoxic effect) and 5000 cells/mL in RPMI media after 72 h (cytostatic effect) of incubation at 37 °C by the MTT assay above. Each experiment was repeated at least three times.

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Keywords:
Lichens · Actinobacteria · Symbiosis · Methacrylate derivatives · Cytotoxicity · Natural products · Structure elucidation
