Lomaiviticins A and B, Potent Antitumor Antibiotics from *Micromonospora lomaivitiensis*

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An earlier study on the marine ascidian *Polysyncraton lithostrotum* resulted in the isolation of a potent antitumor compound, namenamicin.[1] Because of the remarkable similarity of its enediyne aglycon to that found in the actinomycete-derived calicheamicins[2] and esperamicins,[3] it was suspected that the real producer of the namenamicin could be a microbial symbiont. Therefore, a number of actinomycetes were isolated from the inner core of the host ascidian to answer the question of compound origin.[4]

Among these actinomycetes, a halophilic strain, *LL*-37I366, was identified as a new species of the genus *Micromonospora* on the basis of its morphological properties and 16S rDNA sequence, and was named "*Micromonospora lomaivitiensis*".[5] The fermentation broth of this organism exhibited potent DNA-damaging activity indicated by the biochemical induction assay (BIA)[6] and was extremely cytotoxic against a panel of cancer cell lines.[7] Using BIA-guided fractionation, two novel dimeric diazobenzofluorene glycosides were isolated and designated lomaiviticins A (**1**) and B (**2**) (Figures 1 and 3). In this paper, the production, isolation, structural elucidation, and biological activity of the new antibiotics are reported.

Lomaiviticins A (**1**) and B (**2**) were produced by fermentation of strain *LL*-37I366 in a seawater medium in the presence of HP20 resin. The pink colored mixture adsorbed on the resin was extracted with acidic acetonitrile and separated by HPLC to afford the pure antibiotics **1** and **2** (experimental and spectroscopic data, see Supporting Information).

The molecular formula of **1** was determined by high-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry to be $C_{68}H_{80}N_6O_{24}$. The initial analysis of the ^{1}H and ^{13}C NMR spectral data in CD_3OD indicated the presence of 34 carbons and 36 unexchangeable protons, which only accounted for half of the carbons and less than half of the protons in the molecular formula. Therefore, **1** was most likely a symmetric dimer.

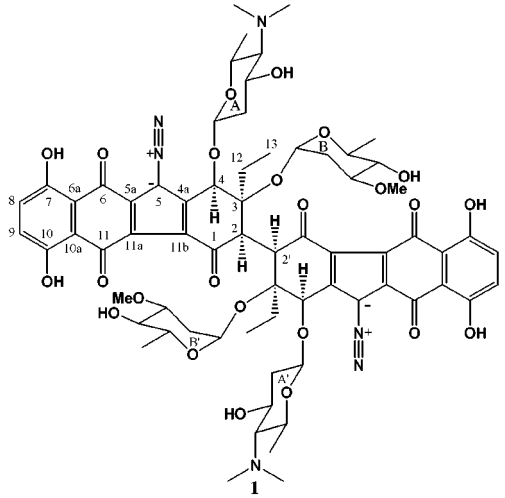

Figure 1. Structure of lomaiviticin A (**1**).

Detailed analysis of NMR spectral data, including 2-D ^{1}H−^{1}H COSY, TOCSY, ^{1}H−^{13}C HMBC, and HMQC spectra, revealed several substructures. The two proton signals of an AX system at δ 7.24 and 7.16 (both d, 9.4 Hz) and 10 carbon signals at 135.1, 186.1, 113.4, 160.4, 131.1, 131.3, 159.4, 114.4, 182.5, and 132.4 were typical for a 5,8-dihydroxy-1,4-naphthoquinone (note the change in numbering from lomaiviticins). The three-bond ^{1}H−^{13}C correlations observed in an HMBC spectrum, together with the extended UV absorption at λ_{max} 525 nm, supported the presence of the substructure. In addition, the COSY and TOCSY data delineated two sugar moieties, A and B. For moiety A, the anomeric proton at δ 4.46 was coupled to H_2-2A at δ 1.93 and 1.50, which were in turn coupled to H-3A at δ 3.88. H-3A was further coupled to H-4A at δ 2.71, and H-4A was coupled to H-5 at δ 3.58, which was coupled to H_3-6A at δ 1.76. The placement of the *N*,*N*-dimethylamino group at C-4A was made by HMBC correlation between the H-4A proton signal at δ 2.71 and $N(CH_3)_2$ carbon signals at δ 43.0. For moiety B, the ^{1}H−^{1}H spin system was identified by analysis of COSY spectral data and evidence for a 3-OCH_3 was found in an HMBC correlation between the proton signal at δ 3.60 (3H, s) and the C-3B resonance at δ 79.8. The relative stereochemistry of the sugar moieties was determined by homonuclear coupling constants. For moiety A, the coupling constants between H-1A and H_2-2A were 9.5 and 1.6 Hz, respectively, requiring an axial orientation for the anomeric proton H-1A. On the other hand, the large couplings (9.5 Hz) between H-3A and H-4A, and between H-4A and H-5A indicated a 3,5-diaxial orientation. The sugar moiety A was thus defined to be the *N*,*N*-dimethyl derivative of the rare amino sugar pyrrolosamine, found in β-pyrrolosporin A.[8] A similar analysis led to the conclusion that moiety B had an equatorial orientation for its anomeric proton H-1B and a 3,5-diaxial configuration for H-3B, H-4B, and H-5B, which was identical to that of α-oleandrose. Obviously, due to the symmetry of the molecule, there should be two identical units of the 5,8-dihydroxy-1,4-naphthoquinone substructure and the sugar moieties A and B (designated A′ and B′).

The center region of the molecule was characterized to be a dimeric cyclohexenone unit. The ^{1}H and ^{13}C NMR signals indicated the presence of an CH_2CH_3 group, two CH's (δ 47.6, 67.3), a C═O (δ 198.4), and three quaternary carbons (δ 83.6, 128.5, 136.1). These elements were pieced together by the ^{1}H−^{13}C correlations in an HMBC spectrum (Supporting Information,

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(2) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGrahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466−68.

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(4) There is a hypothesis that some natural products isolated from the marine invertebrates may be produced by symbiotic microorganisms, because of their structural similarities to compounds of microbial origin. However, this hypothesis has not yet been proven by experimental data. See: Haygood, M. G.; Schmidt, E. W.; Davidson, S. K.; Faulkner, D. J. *J. Mol. Microbiol. Biotechnol*. **1999**, *1* (1), 33−43 and the references therein.

(5) A paper describing taxonomic studies on this species is in preparation.

(6) Greenstein, M.; Wildey, M. J.; Maiese, W. M. The biochemical induction assay and its application in the detection of the calicheamicins. In *Enediyne Antibiot. Antitumor Agents* **1995**, 17−27.

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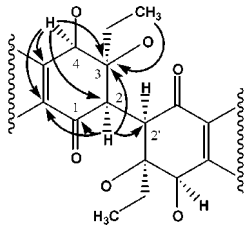

Figure 2. Dimeric 3,4-dihydroxy-3-ethylhexenone substructure in lomaiviticins A (**1**) (selected HMBC correlations are indicated by arrows).

Table 1) to give a 3,4-dihydroxy-3-ethylhexenone. Moreover, in both HMQC and HMBC spectra, the H-2 signal at δ 4.00 was clearly correlated to carbon signals at δ 47.6 (C-2 and C-2′). Since only the multiple-bond $^1H-^{13}C$ correlations were displayed in the HMBC spectrum, a linkage between C-2 and C-2′ was assigned (Figure 2).

The amino sugar moieties A and A′ were required to be connected to C-4 and C-4′ by the HMBC correlations from H-4 and -4′ at δ 5.33 to C-1A and C-1A′ at 95.7. The sugar moieties B and B′ were attached to C-3 and -3′, the only open oxygen-bearing carbons. This was confirmed by ROE's between protons on the ethyl groups at δ 2.21, 2.12, and 1.24 (3H, s) and the anomeric protons, H-1B and 1B′ at δ 6.19. The remaining elements unaccounted for from the molecular formula were four nitrogens and two carbons. The presence of these carbons was shown by broad ^{13}C NMR signals at δ 78.8, which were weakly correlated to H-4 and -4′ in the HMBC spectrum. In addition, an IR band at 2148 cm^{-1}, indicative of triple bonds, was observed. On the basis of NMR and IR data, two diazofluorene moieties were assigned to connect the substructures of the two 5,8-dihydroxy-1,4-naphthoquinones and the dimeric 3,4-dihydroxy-3-ethylhexenone. Lomaiviticin A (**1**) was therefore determined to be a dimeric benzofluorene glycoside attached to two diazo functional groups at C-5 and -5′. The chemical shifts for C-5 and -5′ at δ 78.8 were consistent with literature data for the corresponding carbons in the monomeric diazobenzofluorene antibiotics, kinamycins.[9]

The molecular formula of lomaiviticin B (**2**) was determined to be $C_{54}H_{56}N_6O_{18}$ by high-resolution FTICR mass spectrometry. Similar to lomaiviticin A (**1**), this compound was also considered to be a symmetric dimer. By comparison of its ^1H and ^{13}C NMR data with **1**, compound **2** lacked signals for sugar moieties B and B′, and the ketone signal at δ 198.4 in **1** was replaced by a hemiketal resonance at 96.5 in **2**. Therefore, the center region of compound **2** was established as a fused furanol system (Figure 3), which was supported by $^1H-^{13}C$ correlations in an HMBC spectrum (Supporting Information, Table 2). The remaining portions of the molecule, e.g. the two units of diazobenzofluorene and the sugar moieties A and A′, were determined to be identical to lomaiviticin A (**1**). Compound **2** was probably derived from **1**, by formation of furanol rings between the 3-hydroxyl and 1′-ketone, and between the 3′-hydroxyl and 1-ketone following the hydrolysis of sugars B and B′.

The relative stereochemistry for the center region in lomaiviticin B (**2**) was largely defined by the presence of the fused furanol substructure. To allow the 3(3′)-hydroxyl group to form a five-membered furanol ring with the 1′(1)-ketone group, the ethyl group at C-3(3′), the H-2(2′), and the resulting 1′(1)-hydroxy group had to be synfacial, depicted as down. In lomaiviticin A (**1**), on the other hand, the H-2(2′) signal at δ 4.00 showed a clear cross-peak, attributed to w-coupling, with H-4(4′) at δ 5.33 in the COSY spectrum, which forced H-4(4′) to be down. Here, the configurations of C-2(2′), C-3(3′), and C-4(4′) were respectively assumed to be identical between the two compounds.

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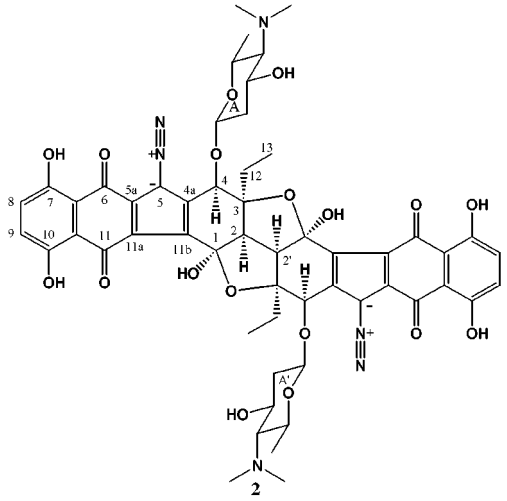

Figure 3. Structure of lomaiviticin B (**2**).

Lomaiviticins A (**1**) and B (**2**) are novel antibiotics containing unique dimeric diazobenzofluorene glycoside structures. They bear some similarities to the kinamycins,[9] the much simpler monomeric diazobenzofluorene antibiotics. The absolute configurations of **1** and **2** were not determined experimentally. However, the center regions of these antibiotics are depicted in a way that the chiralities of C-3, C-3′, C-4, and C-4′ were respectively identical to the corresponding carbons in the kinamycins.

Lomaiviticin A and B were demonstrated to be potent DNA-damaging agents by BIA, both with a minimum induction concentration $\leq$0.1 ng/spot. The more abundant lomaiviticin A was also tested against a number of cancer cell lines and showed cytotoxicity with IC_{50} values ranging from 0.01 to 98 ng/mL (Supporting Information, Table 3). The cytotoxicity profile in the 24-cancer cell line panel[7] was unique compared to the known DNA-damaging anticancer drugs, adriamycin and mitomicin C, suggesting a different mechanism of interaction with DNA molecules. An ongoing study showed that lomaiviticin A cleaved double stranded DNA under reducing conditions. These results will be published elsewhere. Lomaiviticins A and B were also potent antibiotics against Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecium* (MIC's, 6−25 ng/spot) in a plate assay.

In summary, we have isolated two potent antitumor antibiotics, lomaiviticins A and B, with dimeric diazobenzofluorene glycoside structures, from the cultural broth of "*Micromonospora lomaiviticiensis*". Although no namenamicin-like compounds were detected from the fermentation broth of *LL*-37I366, several other actinomycete strains, also isolated from *Polysyncraton lithostrotum*, exhibited DNA-damaging activity, and their active components will be examined. The results described in this paper did not solve the mystery of the namenamicin origin; however, the discovery of the potent antitumor lomaiviticins provides an example of a marine-invertebrate-associated microorganism that is an excellent resource for new bioactive natural products.

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Supporting Information Available: Fermentation and isolation conditions, $[\alpha]_D$, MS, UV, IR, ^1H NMR, ^{13}C NMR, and $^1H-^{13}C$ HMBC spectral data (**1** and **2**), and IC_{50} values for cancer cell lines (**1**) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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