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Antibacterial natural products lobophorin L and M from the marine-derived *Streptomyces* sp. 4506

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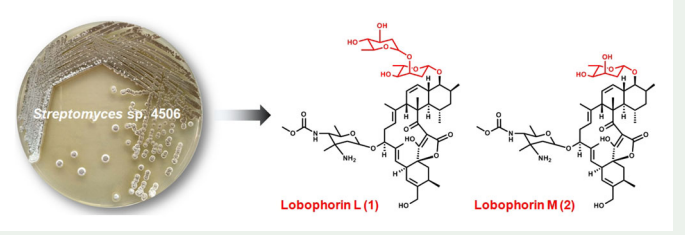
ABSTRACT

Two new spirotetronate natural products, lobophorin L (**1**) and lobophorin M (**2**), together with three known lobophorin-like spirotetronate antibiotics (**3**–**5**) and two known ansamycins (**6**–**7**), were isolated from the marine-derived *Streptomyces* sp. 4506. The structures of **1** and **2** were established on the basis of HRESIMS as well as 1D and 2D NMR datasets. Antibacterial assay showed that, compounds **1** and **3**–**5** exhibited strong to moderate antibacterial activities against *Micrococcus luteus* and *Bacillus thuringiensis* with MIC values ranging from 0.0625 to 8 μg/mL, while compounds **3** and **6** showed weak antibacterial activities against *Staphylococcus aureus* and MRSA. The antibacterial activities of the lobophorins in this study indicated that the more substitution number of the sugar moieties at C-9 of the lobophorin, the stronger antimicrobial properties it may deserve, and the higher the oxidation degree of substituent group at C-3_D, the better antibacterial activities of its corresponding compound could be.



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1. Introduction

Bacterial infection is a serious disease threatening people's health. The steadily rising of the drug-resistant bacteria around the world increased the difficulty of treatment (Lewis 2013; Jimenez 2018). There is an urgent need for new antibiotics to resolve this problem. So more focuses are moving to the abysmal environment, which have novel

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habitats for bacteria with expectation that there would be more new compounds to be discovered (De la Calle 2017; Blunt et al. 2018). In the past years, deep sea-derived microorganism is emerging as the most prolific sources for drugs discovering, and has uncovered lots of nature products exhibiting excellent biological activities (De la Calle 2017), including the antibacterial secondary metabolites (Choudhary et al. 2017).

Streptomyces produces numerous bioactive secondary metabolites such as antifungals, antivirals, antitumorals, anti-hypertensives etc. (Thompson et al. 2002). In which, spirotetronates have many members containing an unusual aglycone with a unique tetronic acid and a trans-decalin system such as tetrocarcin A (Fang et al. 2008), chlorothricin (Jia et al. 2006), kijanimicin (Zhang et al. 2007) and lobophorins (Jiang et al. 1999) etc. Lobophorins (lobophorins A–K and lobophorins CR1–CR3) have been isolated from marine-derived *Streptomyces* (Niu et al. 2011; Wei et al. 2011; Chen et al. 2013; Pan et al. 2013; Brana et al. 2017). Biosynthetic mechanism studies reveal that the aglycone is formed through modular type I polyketide synthases (PKSs) and a glycerate-derived three-carbon unit intra-molecular cyclization (Zhang et al. 2007; Wu et al. 2012). Ansamycins are an important family of macrolactams mainly from *Streptomyces* species with excellent biological activities, for example, antitumor activities of geldanamycins (Fukuyo et al. 2010) and the anti-tubercular activities of rifamycins (Floss and Yu 2005) etc. Ansamycins are also synthesized by modular type I PKSs and start by loading 3-amino-5-hydroxybenzoic acid (AHBA) to the chain-initiation domain. In the end of the backbone biosynthesis, the nascent polyketide chain is released by intra-molecular amidation (Zhao et al. 2015).

Our previous research on bioactive secondary metabolites from the deep sea-derived microorganisms led to the isolation of equisetin with antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Luo et al. 2018). Recently, during our screening of antibiotics from the deep sea-derived actinomycetes, we found that the crude extracts of *Streptomyces* sp. 4506 (a gift from Professor Jianhua Ju) from South China Sea showed antibacterial activities against *Micrococcus luteus* and *Bacillus thuringiensis*. Further chemical investigations of its antibacterial agents found that lobophorin and divergolide-type ansamycin derivatives were responsible for its antimicrobial activities. Here we report the isolation, structure elucidation, and antibacterial activities of the two new compounds lobophorin L (**1**), lobophorin M (**2**) and the five known compounds: lobophorin B (**3**), lobophorin A (**4**), lobophorin CR1 (**5**), divergolide R (**6**) and olimycin B (**7**) (Figure 1).

2. Results and discussion

Lobophorin L (**1**) had a molecular formula of $C_{54}H_{80}N_2O_{16}$ deduced from the protonated ion at *m/z* 1013.5618 ($[M+H]^+$) observed in its the High-Resolution Electrospray Ionization Mass Spectroscopy (HRESIMS) (calcd. for $C_{54}H_{81}N_2O_{16}$, 1013.5581). The IR spectrum of **1** showed characteristic absorptions of hydroxyl (3433 cm^{-1}), methyl (1384 cm^{-1}), methylene (2930 cm^{-1}), carboxyl (1729 cm^{-1}), olefinic groups (1628, 1548 and 1412 cm^{-1}) and carbon oxygen (1060 cm^{-1}). The ^1H, ^{13}C-NMR and HSQC spectra allowed the assignments of the fifty-four carbon signals, including ten methyl, one methoxyl, seven methylene and twenty-five methine and eleven quaternary carbon

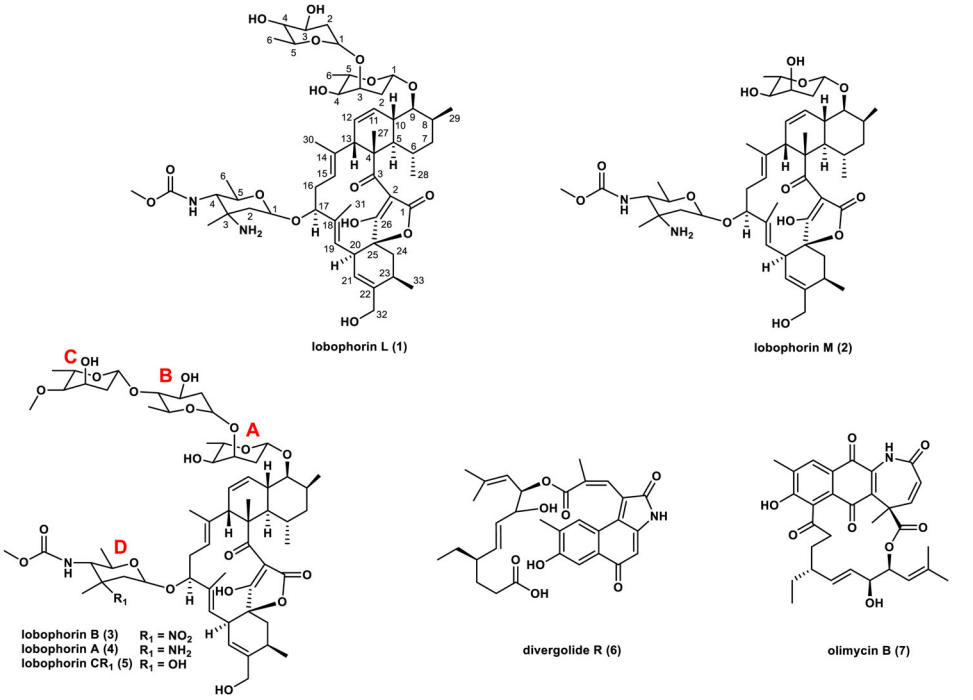

Figure 1. Chemical structures of the lobophorin-like spirotetronates 1–5 and divergolide-type ansamycins 6–7 from *Streptomyces* sp. 4506.

atoms. Among them, the three anomeric carbons (δ_C 99.8, 93.7, 98.1) suggested the presence of three sugar residues. The COSY and HMBC correlations led to the assignment of four spin systems fragments C-5/C-6(C-28)/C-7/C-8(C-29)/C-9/C-10/C-11/C-12/C-13, C-15/C-16/C-17, C-19/C-20/C-21 and C-23(C-33)/C-24. The HMBC correlations of H_3-27 to C-4, C-5 and C-13 assigned a substituted decalin moiety. The HMBC correlations of H_3-30 to C-13, C-14, C-15; H_3-31 to C-17, C-18, C-19; H_2-32 to C-21, C-22, C-23; OH-32 to C-22; H_2-33 to C-22, C-23, C-24 corrective the remaining three fragments as a long fragment of C-14 – C-24. The four carbon signals at δ_C 177.5 (C-1), 99.6 (C-2), 202.9 (C-26), and 85.3 (C-25) suggested a tetronic acid moiety existed in **1**. The HMBC correlations of H-20 to C-24, C-25; H-24 to C-20, C-25; H_3-27 and H-5 to C-3; H_3-30 to C-13, C-15; OH-26 to C-2, C-3, C-25, C-26 indicated the above fragments could be linked to form the characteristic tetronic acid spiro-linked moiety of spirotetronates. Signals observed in its ^{1}H and ^{13}C NMR spectra (Table S1, Supplementary material) and correlations observed in the 2D NMR spectra were indicative of a lobophorin-like spirotetronate structure for **1**. By careful comparison with the NMR data of lobophorin A, we found that **1** only loss a sugar moiety relative to lobophorin A. The molecular weight of 144 mass unit deficiencies relative to lobophorin A (Jiang et al. 1999), indicated the loss of 4-O-methyl-L-digitoxose moiety (sugar C residue). It matched with the MS fragments of **1** at *m/z* 883.4976 ($[M+H]^+$), and *m/z* 753.4354 ($[M+H]^+$) (Figure S1, Supplementary material). Consistently, the correlation of neighboring protons in sugar moieties observed in ^{1}H-^{1}H COSY spectrum showed to be H_{1A}-H_{2A}-H_{3A}-H_{4A}-H_{5A}-H_{6A}, H_{1B}-H_{2B}-H_{3B}-H_{4B}-H_{5B}-H_{6B}, H_{1D}-H_{2D}-H_{3D}-H_{4D}-H_{5D}-H_{6D}; while the key HMBC

correlations detected within the sugar units were as following: H-1_D with C-5_D, C-3_D, C-2_D, C-17; H-1_B with C-5_B, C-3_B, C-2_B, C-3_A; H-3_A with C-1_B, C-5_A, C-2_A, C-1_A; H-1_A with C-5_A, C-3_A, C-2_A, C-9 (Table S1 and Figure S2, Supplementary material). These further confirmed the loss of one sugar moiety relative to lobophorin A. The correlations of H-1_A to C-9, H-9 to C-1_A, H-1_D to C-17, H-17 to C-1_D assigned the points of attachment for the sugar moieties of ring A and ring D to the C-9 and C-17 sites of the aglycone, respectively.

The *Z*-configuration of the $\Delta^{11,12}$ double bond was assigned on the basis of the coupling constant $^{3}J_{\text{H-11/H-12}}$ (10.2 Hz). The ROE correlations of H-13/H-15 and H_3-31/H-20 indicate that both double bonds $\Delta^{14,15}$ and $\Delta^{18,19}$ are in *E*-configurations, respectively. The cross-peaks of H-13/H_3-27/H-10/H-6 placed these protons and methyl groups on the same side of the decalin ring. The ROESY correlations of H-5/H-9 and H-8/H_3-28 in the ROESY spectrum suggested H-5, H-9, H-8, H_3-28 were cofacial (Figure S3, Supplementary material). The relative configuration of anomeric carbon of the sugars was suggested to be α-configuration for sugars A and B and β-configuration for sugar D on the basis of the coupling constants of the corresponding anomeric protons.

According to the HRESIMS analysis, the molecular formula of lobophorin M (**2**) (*m/z* 883.4943 $[M+H]^+$, calcd. for $C_{48}H_{71}N_2O_{13}$, 883.4951) was established as $C_{48}H_{70}N_2O_{13}$. The IR spectrum of **2** showed characteristic absorptions of hydroxyl (3440 cm^{-1}), methyl (1384 cm^{-1}), methylene (2930 cm^{-1}), carboxyl (1730 cm^{-1}), olefinic groups (1628, 1552 and 1414 cm^{-1}) and carbon oxygen (1060 cm^{-1}). In comparison of the ^{1}H and ^{13}C NMR spectra data of **2** with those of **1**, the signals of L-digitoxose moiety (sugar B, Table S1, Supplementary material) in **1** were absent in **2**. Consistent with the loss of this sugar moiety in **2**, only the MS fragment at *m/z* 753.4314 ($[M+H]^+$) of **2** was observed (Figure S4, Supplementary material). In accordance with this change, the COSY and HMBC correlations of the sugar B moiety were absent (Figure S2, Supplementary material). Based on these data, the structure of **2** was assigned and named as lobophorin M.

As with **1**, the relative configuration of **2** was assigned on the basis of the coupling constant and ^{1}H-^{1}H ROESY's correlations. As anticipated, the $\Delta^{11,12}$ double bond was assigned as *Z*-configurations and both double bonds of $\Delta^{14,15}$ and $\Delta^{18,19}$ are *E*-configurations. Correspondingly, H-5, H-9, H-8 and H_3-28 were on the same side, while H-13, H_3-27, H-10 and H-6 are on the other side of the decalin ring (Figure S3, Supplementary material). So the relative configuration of **2** are resemble to that of **1**.

Furthermore, five known compounds: lobophorin B (**3**) (Jiang et al. 1999), lobophorin A (**4**) (Jiang et al. 1999), lobophorin CR1 (**5**) (Cruz et al. 2015), divergolide R (**6**) (Zhao et al. 2015), olimycin B (**7**) (Sun et al. 2018) were identified by comparison of their NMR data with those of literatures.

As to the paradigm of the glycosyltransferases (GTs) of lobophorin, Zhang's lab found that LobG1–LobG3 are responsible for appending four sugars. Further characterization of five differentially glycosylated metabolites from three GT gene-inactivation, they concluded that LobG3 is an iterative GT to attach two digitoxoses (Li et al. 2013). To our surprise, the isolated lobophorin M (**2**) in this study only has one digitoxose. So the function of LobG3 in *Streptomyces* sp. 4506 may be different with that of Zhang's lab, or LobG3 is an iterative GT to attach two digitoxoses step by step, which

need to be further proven. The ansamycins isolated in this paper are all divergolide-type ansamycins. According to the biosynthetic studies, they all fit into one biosynthetic scheme and diverge biosynthetic pathways via generate a reactive precursor, which result in metabolites that differ in their bioactivity profiles (Ding et al. 2011), covering antibacterial and antitumor properties (Li et al. 2015).

Lobophorin-like spirotetronate antibiotics possess various biological activities, such asanti-inflammatory (lobophorins A and B) (Jiang et al. 1999), antibacterial (lobophorins F) (Niu et al. 2011), and antitumor (lobophorins C, D, CR1 and CR2) (Wei et al. 2011; Cruz et al. 2015) activities. Considering that both lobophorins and ansamycins showed antimicrobial activities, we further examined compounds **1**–**7** for antimicrobial activities against a panel of bacteria using the previous reported method (Luo et al. 2018; Song et al. 2020). Results showed that compounds **1** and **3**–**5** showed strong to moderate antibacterial activities against *Micrococcus luteus* and *Bacillus thuringiensis* with minimum inhibitory concentration (MIC) values ranging from 0.0625 to 8 μg/mL, while compounds **3** and **6** showed weak antibacterial activities against *Staphylococcus aureus* and MRSA (Table S2, Supplementary material). By comparison with the MIC values of lobophorins (compounds **1**, **2**, **3**, **4** and **5**) isolated in this study, we found that both the substitutions at C-9 and C-3_D of the lobophorins affected its antimicrobial activities. The more substitution number of the sugar moieties at C-9, the stronger antimicrobial properties it may have, which was agree with experiments of inactivates kijanimicin by the hydrolytic removal of sugar residues (Leslie et al. 2013), and the higher the oxidation degree of substitution at C-3_D, the better the antibacterial activities of its corresponding compound could be. The three sugar moieties substitution in compound **4** (MIC of 0.25 μg/mL) showed the strongest antibacterial activities compared with compounds **1** (two sugar moieties substitution with MIC of 8 μg/mL) and **2** (one sugar moiety substitution with MIC above 128 μg/mL) against *Micrococcus luteus*. It is also consistent with common recognition that the diversity of structures and bioactivities depends on decoration of aglycones with deoxysugar and/or other peripheral moieties.

3. Conclusions

In conclusion, five lobophorins and two divergolide-type ansamycins, including two new antimicrobial natural products lobophorin L (**1**) and lobophorin M (**2**) were isolated from the deep-sea derived strain *Streptomyces* sp. 4506. The structures of **1**–**2** were established by HRESIMS and NMR analyses. Compounds **1** and **3**–**5** exhibited strong to moderate antibacterial activities against *Micrococcus luteus* and *Bacillus thuringiensis* with MIC values ranging from 0.0625 to 8 μg/mL. Compounds **3** and **6** showed weak antibacterial activities against *Staphylococcus aureus* and MRSA. The antibacterial activities of the lobophorins in this study indicated that the more substitution numbers the sugar moieties at C-9 of the lobophorin, the stronger antimicrobial properties it may perform, and the higher oxidation degree of substituent group at C-3_D, the better the antibacterial activities of its corresponding compound could be.

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Disclosure statement

The authors declare no competing financial interest.

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