Studies on Saccharothriolides A-C, Novel Phenyl-substituted 10-Membered Macrolides from a Rare Actinomycete Saccharothrix sp.

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In the course of our chemical screening for novel microbe metabolites, we discovered three new 10-membered macrolides, saccharothriolides A-C (1-3), from a rare actinomycete Saccharothrix sp. A1506 (Figure 1). Here we present the isolation, structure elucidation, and biological activities of these new macrolides.¹

1. Fermentation, extraction, and isolation.

Saccharothrix sp. A1506 was isolated from a soil sample collected in Yamanashi Prefecture, Japan. The culture broth (6 L) was extracted with n-BuOH to afford a residue (5.44 g). The residue was subjected to column chromatography on silica gel and then subjected
to RP-HPLC to yield metabolites 1 (24.7 mg), 2 (5.4 mg), and 3 (17.8 mg).

2. Structure elucidation of saccharothriolides A-C (1-3).

Saccharothriolide A (1) was obtained as a light yellow oil with $[\alpha]_D^{20} +18.0$ ($c = 0.74$, MeOH). The molecular formula was determined to be C$_{26}$H$_{31}$NO$_8$ by HR-ESI-MS. The HMQC spectrum facilitated the assignment of the protons to the corresponding carbon atoms, and the H-1H COSY spectrum gave three proton sequences (Figure 2, left). The observation of HMBC correlations led to the formation of the 10-membered lactone ring (Figure 2, left). The relative stereochemistry of metabolite 1 was deduced from the NOESY data to be 2R*, 3R*, 4S*, 6R*, 7R*, 8R*, 9S* (Figure 2, right).

![Diagram](image-url)

Figure 2. H-1H COSY (left, bold) correlations and selected HMBC (left, arrow) and NOESY (right) correlations in saccharothriolide A (1)

The relative stereochemistry of 1 were further confirmed by the advanced statistical Universal NMR Database (UDB) approach. The $^{13}$C NMR spectroscopic data for the two C5–C2 and C3–C6 segments of 4 which was obtained by reduction of 1 were subjected to statistical analysis. The analysis suggested an anti-anti-anti configuration, which was in good agreement with the NOESY data in
In order to determine the absolute configuration of the secondary C-3 hydroxyl group by the Mosher's method (Figure 4), phenolic hydroxyl groups and the carboxylic acid of 1 were first protected by methylation with CH$_3$I to yield a tri-methyl derivative 5. The $\Delta\delta$ ($\delta_S-\delta_R$) values of (S)- and (R)-MTPA esters of 5 for the protons flanking the C-3 chiral center revealed the 3$R$ absolute configuration, which in turn concluded the absolute configurations of 1 as 2$R$, 3$R$, 4$S$, 6$R$, 7$R$, 8$R$, 9$S$. The result was confirmed by comparing the measured CD spectrum of 1 and the electronic circular dichroism (ECD) spectrum calculated by time-dependent density functional theory (TDDFT) (Figure 5).
Saccharothriolide B (2) was obtained as a light yellow oil. Its molecular formula of C_{25}H_{31}NO_{7} was established by HR-ESI-MS. The $^1$H and $^{13}$C NMR data of 2 were very similar to those of 1, suggesting that 2 possessed a phenolic hydroxyl group instead of a carboxylic acid at C-2". The planar structure was deduced by the COSY, HMQC and HMBC data. Detailed analysis of the NOESY spectrum of 2 revealed that the relative stereochemistry was the same as that of 1. The absolute stereochemistry of 2 was determined by measurement and calculation of ECD as same as 1.

Saccharothriolide C (3) was also obtained as a light yellow oil, while the molecular formula was determined to be C_{19}H_{26}O_{7} by HR-ESI-MS. The $^1$H and $^{13}$C NMR data of 3 were similar to those of 1 and 2. Instead of the amino aryl groups substituted at C-7 in 1 and 2, metabolite 3 possessed a hydroxyl group at C-7. The planar structure was elucidated by detailed analysis of the 2D NMR data, while the NOESY data revealed the same relative configuration with those of 1 and 2. The absolute stereochemistry of 3 was the same as those of 1 and 2, which was determined by the ECD analysis (Figure 5).


The growth inhibitory activity of saccharothriolides 1-3 toward the cancer cell lines of HeLa and HT1080 was evaluated. Saccharothriolide B (2) showed moderate cytotoxicity against cancer cells including HeLa and HT1080 cell lines with IC_{50} values of 17.9 and 13.9 μM, respectively. Other saccharothriolides 1 and 3 were inactive even at 100 μM. Additionally, only metabolite 2 showed weak antibacterial activity at 50 μg per disc against *Staphylococcus*
aureus in a paper disc assay.

4. Plausible biosynthetic pathway of saccharothriolides A-C (1-3).

We also hypothesized the polyketide biosynthetic pathway of saccharothriolides A-C (1-3): an aryl starter unit and four units of methyl-malonyl-CoA seem to be conjugated followed by cyclization to yield the precursor metabolite A. The precursor A with α,β-unsaturated ketone is likely attacked by an aminoaryl group to furnish metabolites 1 and 2, or by a water molecule to furnish metabolite 3 (Scheme 1).

![Diagram of biosynthetic pathway]

Scheme 1. Plausible biosynthetic pathway of saccharothriolides A-C (1-3).

References


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Novel Phenyl-substituted 10-Membered Macrolides
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We surveyed more than 30,000 microbe cultures by LC-MS
analysis to find novel metabolites, and isolated three new
10-membered macrolides, saccharothriolides A-C (1-3), from a rare
actinomycete *Saccharothrix* sp. A1506.

Their chemical structures were deduced by extensive
spectroscopic analysis including advanced universal NMR database
method. The absolute configurations were determined by the
modified Mosher’s method and TDDFT-calculation of ECD spectra.
Saccharothriolides A (1) and B (2) had an aminoaryl substituent in
the lactone ring through a C-N bond, while saccharothriolide C (3)
possessed a hydroxyl group at C-7. Biogenetically, these three new
metabolites 1-3 might be generated from the precursor metabolite A.

Saccharothriolide B (2) exhibited moderate cytotoxicity against
human tumor cell lines HeLa and HT1080, and showed weak
antibacterial activity against *Staphylococcus aureus* in a paper disc
assay.