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Saccharothriolides A–C, novel phenyl-substituted 10-membered macrolides isolated from a rare actinomycete *Saccharothrix* sp.†

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Three new 10-membered macrolides, saccharothriolides A–C (1–3), were discovered from a rare actinomycete *Saccharothrix* sp. A1506. All of the sp^3 carbons in the 10-membered ring had chirality, which was determined by extensive spectroscopic analysis and TDDFT-calculation of ECD spectra. Saccharothriolide B (2) exhibited cytotoxicity against human tumor cell lines HeLa and HT1080.

Rare actinomycetes (or non-streptomycete actinomycetes) are regarded as the actinomycete strains whose isolation frequency determined by conventional methods is much lower than that of the streptomycete strains. These under-explored microorganisms are expected to be a rich source of natural products with novel chemical structures and biological activities.[1,2] *Saccharothrix* sp., one of the rare actinomycetes, was first obtained from a soil sample collected in Australia in 1984.[3] Dozens of bioactive natural products with a structural and biological diversity have been isolated from this genus: for example, a naphthoquinone derivative sacchathridine A which was reported as a prostaglandin release inhibitor,[4] 16-membered macrolides tianchimycins A and B,[5] cytotoxic 20-membered macrolides ammocidins A–D,[6,7] antibacterial galacardins A and B,[8] and antitumor rebeccamycin.[9] In the course of our chemical screening for novel microbe metabolites, we discovered new 10-membered macrolides from a rare actinomycete *Saccharothrix* sp. A1506, designated as saccharothriolides A–C (**1**–**3**) (Fig. 1). Here we report their isolation, structure elucidation, and biological activities.

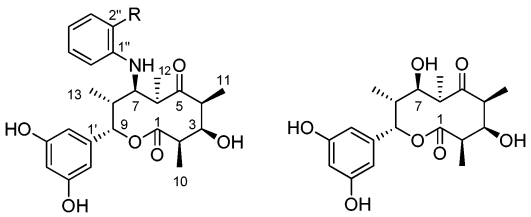

Fig. 1 Chemical structures of saccharothriolides A–C (**1**–**3**).

We surveyed more than 30 000 microbe cultures by LC-MS analysis to identify a novel metabolite from a culture broth of a rare actinomycete *Saccharothrix* sp. A1506. This metabolite, named saccharothriolide A (**1**), exhibited an MS signal (*m/z* 486.2134 $[M + H]^+$) which was not found in metabolites database and a UV absorption at 345 nm characteristic to anthranilic acid. Further analysis of the LC-MS data revealed the presence of two congeners, saccharothriolides B (**2**) and C (**3**). By LC-MS-guided isolation, we purified metabolites **1** (24.7 mg), **2** (5.4 mg), and **3** (17.8 mg) from a 6 L-culture.

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 (*m/z* 486.2134 $[M + H]^+$, calcd 486.2128), indicating the presence of 12 degrees of unsaturation. The IR spectrum showed absorptions corresponding to hydroxyl (3327 cm^{-1}), ester carbonyl (1732 cm^{-1}), and ketone (1679 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed seven aromatic protons (δ_H 7.95, 7.31, 6.66, 6.57, 6.06, 5.87 × 2 ppm) and two oxymethine protons (δ_H 5.50, 3.79 ppm) in addition to four aliphatic methyl signals (δ_H 1.09, 1.20, 1.35, 1.46 ppm) and five methine protons (δ_H 2.19, 2.88, 3.28, 3.43, 3.66 ppm) (Table 1). The ^{13}C NMR spectrum included 26 carbon signals, corresponding to four aliphatic methyls (δ_C 10.7, 14.1, 18.4, 20.3), seven aliphatic methines including two carbons adjacent to an oxygen atom (δ_C 74.3 and 81.4) and one carbon adjacent to a nitrogen atom (δ_C 62.2), twelve aromatic carbons and three carbonyl carbons (δ_C 173.8 × 2, 224.6).

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Table 1 ^{1}H and ^{13}C NMR data of saccharothriolide A (**1**) in methanol-d_4

	1	
No.	δ_H, m, J (Hz)	δ_C
1	—	173.8, C
2	2.88, qd, 6.9, 3.4	46.3, CH
3	3.79, brs	81.4, CH
4	3.28, q, 7.5	50.8, CH
5	—	224.6, C
6	3.43, q, 6.9	43.7, CH
7	3.66, brs	62.2, CH
8	2.19, qd, 6.9, 5.2	43.1, CH
9	5.50, brs	74.3, CH
10	1.20, d, 6.9	14.1, CH_3
11	1.46, d, 7.5	18.4, CH_3
12	1.35, d, 6.9	20.3, CH_3
13	1.09, d, 6.9	10.7, CH_3
1′	—	144.9, C
2′,6′	5.87, s	104.7, CH
3′,5′	—	159.5, C
4′	6.06, s	102.3, CH
1″	—	151.7, C
2″	—	113.7, C
3″	7.95, brs	134.1, CH
4″	6.57, t, 5.7	115.6, CH
5″	7.31, t, 7.5	135.6, CH
6″	6.66, d, 8.6	111.8, CH
2″-COOH	—	173.8, C

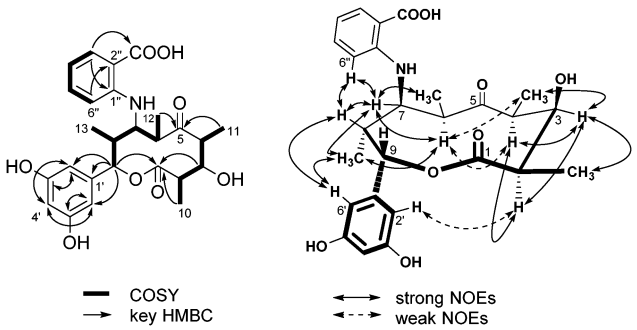


Fig. 2 ^{1}H–^{1}H COSY (left, bold) correlations and selected HMBC (left, arrow) and NOESY (right) correlations in saccharothriolide A (**1**).

The ^{1}H–^{1}H COSY experiment revealed the presence of three spin systems: CH_3-10/H-2/H-3/H-4/CH_3-11, CH_3-12/H-6/H-7/H-8/CH_3-13/H-9, and H-3″/H-4″/H-5″/H-6″ (Fig. 2). HMBC correlations from CH_3-11 to C-5, and from CH_3-12 to C-5 connected C-4 and C-6 through a ketone group. HMBC correlations from both H-3 and H-9 to carbonyl C-1 connected C-2 and C-9 through an ester bond, leading to the formation of the 10-membered lactone ring (Fig. 2). The formation of the lactone ring was further supported by the observation of the down-field shifted chemical shift for H-9 (δ_H 5.50). The *meta*-disubstituted benzene ring was determined on the basis of the HMBC correlations from H-4′ to C-2′/C-6′, and from H-2′/H-6′ to C-3′/C-5′. This benzene ring was connected to C-9 due to the HMBC correlations from H-9 to aromatic carbons C-1′ and C-2′/6′. The presence of anthranilic acid suggested by the characteristic UV absorption (Fig. S6, ESI†)[10] was confirmed by HMBC correlations from H-6″ (δ_H 6.66) to C-2″ (δ_C 113.7), and from H-3″ (δ_H 7.95) to C-1″ (δ_C 151.7) and 2″-COOH (δ_C 173.8), along with the ^{1}H–^{1}H COSY correlations from H-3″ to H-6″ (Fig. 2). The anthranilic acid was connected to the lactone ring at C-7 through an NH group, which was deduced by the up-field shifted chemical shift for C-7 (δ_C 62.2) and the presence of a free carboxylic acid (δ_C 173.8). This connection was further confirmed by the mutual NOESY correlations among H-6″, H-7, and H-8 (Fig. 2).

The relative stereochemistry of metabolite **1** was deduced from the NOESY data (Fig. 2). NOESY cross peaks between H-2 and H-4 indicated that they are placed in the same α face, whereas NOESY correlations between H-3 and H-2, H-4, CH_3-10, and CH_3-11 suggested α configuration for H-3. NOESY correlations between H-6 and H-4(weak), CH_3-11(weak), and CH_3-13 indicated an α configuration for these protons. A β configuration for H-8 was then determined. Correlations between H-7 and H-6, H-8, CH_3-12, and CH_3-13 suggested that H-7 has an α configuration. Finally, NOESY correlations between the aromatic proton H-6′ and H-8, and CH_3-13, together with a weak correlation from the aromatic proton H-2′ to H-2, revealed the β orientation of H-9. Thus, the relative configurations were deduced to be 2*R**, 3*R**, 4*S**, 6*R**, 7*R**, 8*R**, 9*S**.

Macrolides can exist in several conformations, which can be a potential cause of mis-interpretation of the NOESY data. We next analyzed the relative stereochemistry of **1** by the advanced statistical Universal NMR Database (UDB) approach, originally developed by Kishi and co-workers.[11–13] Reductive opening of the lactone ring of **1** using $LiAlH_4$ furnished a linear product **4** (Fig. 3). The ^{13}C NMR data of the two tetrad segments, C3–C6 and C5–C2, were subjected to the statistical UDB analysis. The difference between the adjusted NMR data of the tetrad segments in **4** and Kishi's database was calculated to reveal that both sequences have an *anti–anti–anti* configuration (Table S2–S5, ESI†), which was in good agreement with the NOESY data in **1**.

The absolute stereochemistry was determined by the modified Mosher's method (Fig. 4).[14] Phenolic hydroxy groups and carboxylic acid of **1** were first protected by methylation using CH_3I to yield a tri-methyl derivative **5**. The methylated derivative **5** was treated with (*R*)- and (*S*)-MTPA chloride to afford (*S*)- and (*R*)-MTPA esters of **5**, respectively. The $\Delta\delta$ (δ_S–δ_R) values of 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 modified Mosher's method is sometimes not applicable to axial hydroxyl groups.[15] In order to confirm the above results,

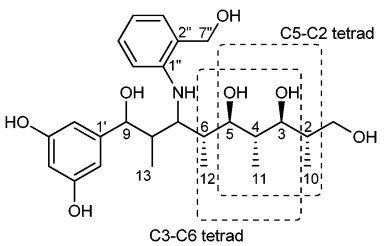


Fig. 3 Analysis of the stereochemistry of compound **4** (C3–C6 and C5–C2 tetrads) by the statistical UDB approach.

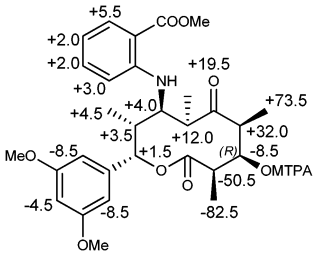


Fig. 4 $\Delta\delta$ (δ_S–δ_R) values (in Hz) for the MTPA esters of **5**.

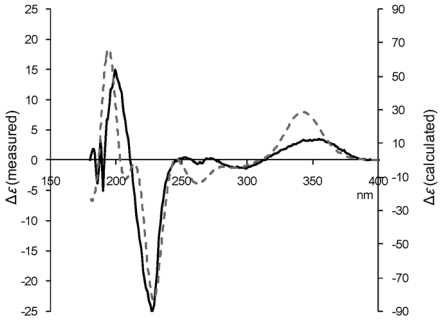


Fig. 5 Experimental CD (solid line) and calculated ECD (dotted line) spectra of saccharothriolide A (**1**). ECD was calculated for 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S*.

we finally measured the CD spectrum of **1**, which was compared with the electronic circular dichroism (ECD) spectrum calculated by the time-dependent density functional theory (TDDFT).[16] As shown in Fig. 5, the experimental CD spectrum showed a Cotton effect at 199 ($\Delta\varepsilon$, +15.0), 227 ($\Delta\varepsilon$, −25.0) and 355 ($\Delta\varepsilon$, +3.46) nm, all of which were observed in the ECD spectrum calculated for the stereochemistry of 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S*. Thus, the absolute configuration of **1** was unambiguously established by two independent methods.

To find congeners of saccharothriolide A (**1**), we investigated the LC-MS data of the crude broth extract. We detected a metabolite whose ion peak was observed at *m*/*z* 458.2162 ($[M + H]^+$), revealing the molecular formula of $C_{25}H_{31}NO_7$. This metabolite, designated as saccharothriolide B (**2**), contained one nitrogen atom similar to **1**, but its molecular size was 28 Da smaller than **1**. Metabolite **2** was obtained as a light yellow oil with $[\alpha]_D^{20}$ −81.2 (*c* = 0.36, MeOH).

The ^{1}H and ^{13}C NMR data of **2** were very similar to those of **1** (Table S1, ESI†), with the exception of the disappearance of the carboxylic acid. The obvious downfield shift of C-2″ (δ_C 113.7 in **1**, δ_C 146.0 in **2**) and an upfield shift of C-1″ and C-3″ (δ_C 151.7 in **1**, δ_C 138.2 in **2** and δ_C 134.1 in **1**, δ_C 115.2 in **2**, respectively) were observed, accompanied by a marked upfield shift of H-3″ and H-5″ (δ_H 7.95 in **1**, δ_H 6.73 in **2** and δ_H 7.31 in **1**, δ_H 6.70 in **2**, respectively), which suggested that **2** possessed a phenolic hydroxyl group instead of a carboxylic acid at C-2″. This was in agreement with the fact that **2** was 28 Da smaller than **1**. The planar structure was deduced by the COSY, HMQC and HMBC data (Fig. S1, ESI†). Detailed analysis of the NOESY spectrum of **2** revealed that the relative stereochemistry was the same as that of **1** (Fig. S1, ESI†): 2*R**, 3*R**, 4*S**, 6*R**, 7*R**, 8*R**, 9*S**.

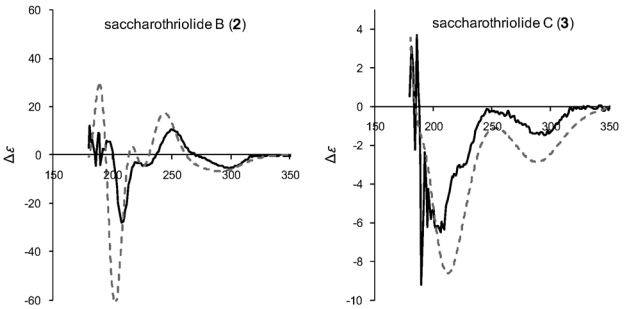


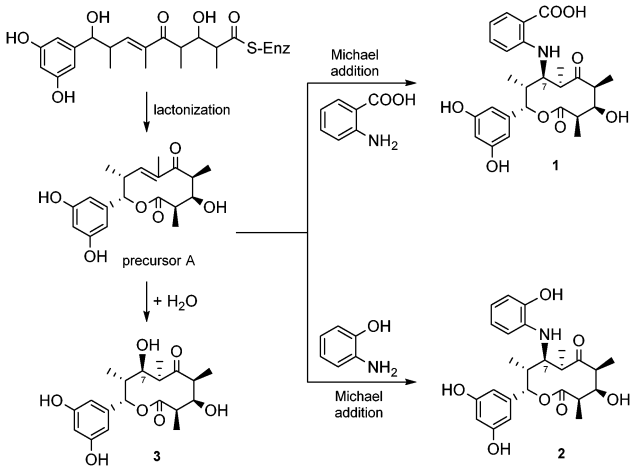
Fig. 6 Experimental CD (solid line) and calculated ECD (dotted line) spectra for saccharothriolides B (**2**) and C (**3**). Both ECD spectra were calculated for 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S*.

The absolute stereochemistry of **2** was determined by measurement and calculation of ECD. The ECD calculation of **2** revealed that the absolute stereochemistry was the same as that of **1**. The calculated ECD spectrum overlapped well with the experimental CD spectrum (Fig. 6), concluding that the absolute stereochemistry of **2** was 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S*.

Saccharothriolides A (**1**) and B (**2**) have an aminoaryl substitution at C-7, suggesting the presence of a common precursor for them, *i.e.*, Michael addition of nucleophilic amines to the corresponding precursor can afford metabolites **1** and **2**. For investigating this possibility, we surveyed the LC-MS data of the culture broth to identify saccharothriolide C (**3**). Metabolite C (**3**) was obtained as a light yellow oil with $[\alpha]_D^{20}$ −111.8 (*c* = 0.58, MeOH). The HR-ESI-MS data indicated an $[M + Na]^+$ ion peak at *m*/*z* 389.1586 ($[M + Na]^+$, calcd 389.1576), revealing a molecular formula of $C_{19}H_{26}O_7$. The ^{1}H and ^{13}C NMR data of **3** were similar to those of **1** and **2** (Table S1, ESI†), except for the absence of a set of aromatic protons and carbon signals corresponding to the amino aryl groups substituted at C-7. Instead, metabolite **3** possessed a hydroxyl group at C-7, supported by the downfield shift of the carbon signal of C-7 (δ_C 80.1). The planar structure was elucidated by detailed analysis of the 2D NMR data, while the NOESY data indicated the same relative configuration as those of **1** and **2** (Fig. S2, ESI†). As expected, the absolute stereochemistry of **3** was the same as those of **1** and **2**, because the calculated ECD spectrum for the stereochemistry of 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S* showed a high similarity to that of the measured one (Fig. 6).

Metabolite **2** exhibited 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. These results indicate that saccharothriolides are capable of regulating their biological activities by modifying the functional group at the C-7 position.

Saccharothriolides are 10-membered macrolides. As in the case of other macrolides, saccharothriolides seem to be synthesized *via* the polyketide biosynthetic pathway (Scheme 1). An aryl starter unit and four units of methyl-malonyl-CoA seem to be conjugated followed by cyclization to yield the precursor



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

metabolite A. The aromatic unit-priming polyketide system has been found only in a limited number of metabolites, *i.e.* soraphen A from *Sorangium cellulosum*,[17] rifamycins from *Amycolatopsis rifamycinica*[18] and enterocin from *Streptomyces maritimus.*[19] The precursor A is likely attacked by an aminoaryl group to furnish metabolites **1** and **2**, or by a water molecule to furnish metabolite **3** (Scheme 1). In fact, the culture broth included a significant amount of anthranilic acid (23.5 mg) and 2-hydroxyacetanilide (120.9 mg).[20] Although we explored the presence of precursor A using the LC-MS data, ion peaks corresponding to the precursor were not found, probably due to the high reactivity of the α,β-unsaturated ketone.

In conclusion, we discovered three novel 10-membered macrolides from a rare actinomycete *Saccharothrix* sp. A1506. Structural analysis implied their common biosynthetic origin, whereas we predict the presence of precursor A possessing α,β-unsaturated ketone. It should be noted that only saccharothriolide B (**2**) showed moderate biological activities. Detailed studies on the biosynthetic mechanism and modes of action are currently being undertaken in our laboratory.

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