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Hainanmycin A, a cyclo-heptadeca macrolide bearing a cyclopentenone moiety from the mangrove-derived *Streptomyces* sp. 219807

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ABSTRACT

A macrocyclic polyketide with novel carbon skeleton, namely hainanmycin A, was isolated from the mangrove-derived *Streptomyces* sp. 219807. This polycyclic compound featured a cyclo-heptadeca framework possessing a 5,6-disubstituted-4-hydroxy-2-pyrone moiety in conjunction with a 2-cyclopentenone residue. The structure was established by spectroscopic analyses, and the stereochemistry was determined by NMR chemical shifts calculations and time-dependent density functional theory (TDDFT) calculation of electronic circulardichroism (ECD). A possible biogenetic pathway for hainanmycin A was proposed.

Introduction

The fifteen or seventeen membered carbocyclic polyketide macrolides featuring a characteristic 6-membered β-keto-δ-lactone (or it's enol form) moiety compose a structurally unique family of metabolites,[1,2] which are different from the macrocyclic rings of common macrolide antibiotics or ansamycins and polyenes. About 20 natural products of this polycyclic polyketide macrolides have been reported to be detected only in the actinomycetes since the 1960s.[1,2] The existing compounds could be subdivided into two categories based on their structural differences: lankacidins,[1] which have a cycloheptadecane skeleton with a lactamide or pyruvamide side chain, and mangromicins[2] (including akaeolide[2d]), which have a cyclopentadecane skeleton with a tetrahydrofuran unit. Previous researches suggested that those compounds demonstrated a wide range of bioactivities such as antimicrobial,[3] antitumor,[4] antitrypanosomal,[2a] anti-oxidative activities,[2b] and even been used commercially for the livestock industries.[5]

In the past decades, mangrove actinomycetes particularly the genus of *Streptomyces* have been recognized as a highly prolific source of novel and biologically active secondary metabolites of high value for drug development.[6] During our ongoing search for structurally diverse and bio-active secondary metabolites from the rich and unique actinomycetes of mangrove origin,[7] the potential strain *Streptomyces* sp. 219807, isolated from mangrove soil collected in Sanya, was selected for investigation after using HPLC-UV profile analysis, HR-MS match in the AntiBase 2012[8] and cytotoxicity testing of fermentation samples as screening strategies. We have recently examined the EtOAc extracts of the culture supernatant of *Streptomyces* sp. 219807 and obtained a new polyketide macrolide hainanmycin A (Fig. 1), which featured an unprecedented structural skeleton of a 17-membered carbocyclic framework fused with a 5,6-disubstituted-4-hydroxy-2-pyrone moiety along with a 5-enol-2-methyl-2-cyclopentenone unit, while the α,β-cyclopentenone portion in conjunction with a enol group is quite rare in nature. Herein, detailed structure elucidation and possible biogenetic pathway of the compound are reported.

Results and discussion

Hainanmycin A was obtained as yellow oil. The molecular formula was established as $C_{21}H_{24}O_5$ (9 degrees of unsaturation) according to the pseudomolecular ion at *m/z* 357.1693 $[M+H]^+$ (calcd 357.1696) detected by HRESIMS. The UV absorptions (236 and 253 nm) together with the IR peaks at the double-bond region (1695, 1664, 1626 cm^{-1}) indicated an α, β-unsaturated lactone functionality[1a,9] and an $\alpha,\beta,\delta,\gamma$-conjugated ketone[10] in the molecule. The ^1H NMR spectrum (Table 1) exhibited three methyl units [δ_H 1.38 (d, J = 7.0 Hz, H-19), 1.68 (s, H-20), 1.78 (t, J = 1.6 Hz, H-21)], an oxymethine [δ_H 4.69 (s, H-5)], along with four olefinic protons [δ_H 5.45 (t, J = 6.8 Hz, H-7), 7.27 (dd, J = 1.3, 2.1 Hz, H-15), 6.72

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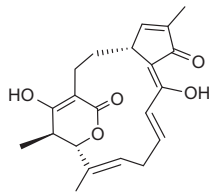

Fig. 1. Structure of hainanmycin A.

Table 1
NMR data of hainanmycin A (CD_3OD, 500 MHz).

No.	δ_C	δ_H mult (*J*, Hz)	HMBC
1	170.9 C		
2	103.7 C		
3	174.9 C		
4	35.3 CH	3.00, q (6.6)	2, 3, 19
5	83.9 CH	4.69, s	1, 3, 4, 6, 7, 19, 20
6	138.8 C		
7	123.1 CH	5.45, t (6.8)	5, 8, 20
8α	31.3 CH_2	2.93, dt (4.4, 19.5)	9
8β		3.09 overlapped	
9	139.1 CH	6.72, dt (4.1, 15.7)	7, 11
10	123.8 CH	5.84, d (15.7)	8, 9, 11, 12,
11	160.8 C		
12	116.4 C		
13	201.8 C		
14	141.9 C		
15	156.3 CH	7.27, dd (1.3, 2.1)	11, 12, 13, 14, 16, 21
16	41.8 CH	3.09 overlapped	12, 13, 15, 17, 18
17α	35.3 CH_2	1.45, m	2, 15, 16, 18
17β		1.65, m	
18	21.4 CH_2	2.45, m	1, 2, 3, 16, 17
19	18.1 CH_3	1.38, d (7.0)	3, 4, 5
20	14.6 CH_3	1.68, s	5, 6, 7
21	10.5 CH_3	1.78, t (1.6)	13, 14, 15

(dt, *J* = 4.1, 15.7 Hz, H-9), 5.84 (d, *J* = 15.7 Hz, H-10)]. Furthermore, the ^{13}C-DEPT NMR spectra (Table 1) perfectly matched the ^{1}H NMR data and differentiated 21 carbons into three methyl groups, three methylenes units, three aliphatic methines groups including an oxygenated one, eight olefinic carbons (four of which are protonated), along with one ketone carbon and three carbonyl and/or enol carbons. All the moieties aforementioned accounted for 7 double bond equivalents, indicating that the remainder 2 degrees of unsaturation were bicyclic to satisfy the molecular formula.

Interpretation of ^{1}H–^{1}H COSY spectrum, two four-carbon fragments were observed: H-15/H-16/H-17/H-18 and H-7/H-8/H-9/H-10 (Fig. 2). The obvious HMBC correlations (Fig. 2) from H-16 to C-12, C-13 and C-15, H-15 to C-11, C-12, C-13, C-14 and C-16, H-21 to C-13, C-14 and C-15 evidenced the presence of a 2-cyclopentenone ring with a methyl group H-21 at C-14 and an enol unit at C-12 (part A in Fig. 2). Moreover, despite the fact that both H-4 and H-5 appeared no recognizable vicinal coupling and ^{1}H–^{1}H

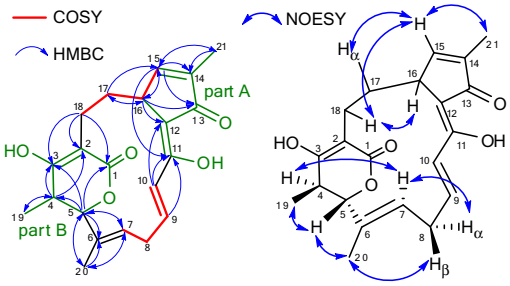

Fig. 2. Key COSY, HMBC and NOESY correlations of hainanmycin A.

COSY signals, the HMBC correlations from H-4 to C-2 and C-3, H-5 to C-1, C-3 and C-4 could jointly prove to be a six membered α, β-unsaturated-δ-lactone ring (part B) (Fig. 2). Besides, the HMBC from methyl unit H-19 to C-3, C-4 and C-5 indicated that H-19 was situated at C-4 position.

Further inspection of 2D NMR data was conducted to assemble of all the substructures (Fig. 2). The connectivities from C-17 to part A at C-16 and from C-18 to part B at C-2 were established by HMBC correlations from H-17 to C-15, C-16 along with H-18 to C-1, C-2 and C-3, respectively. The key HMBC correlations of H-10/C-11, C-12, and H-9/C-11 revealed the attachment of C-10 to the neighboring C-11. Similarly, the correlations from H-5 to C-6 and C-7, H-7 to C-5 in the HMBC spectrum illustrated that the C-6 linked at C-5 in part B. What's more, a CH_3-20 was located at C-6 by the mutual HMBC cross-peaks between H-20 and C-5, C-6, C-7. Taking into account previous observations, the remaining two unassigned degrees of unsaturation could be ascribed to the five-membered cyclic of cyclopentenone and the macrocyclic skeleton to form the entire molecule. Thus, the planar structure was elucidated and named as hainanmycin A (Fig. 2).

The relative stereochemistry at the chiral centers C-4/C-5 as 4*S**, 5*R** was confirmed by coupling constant data and NOESY experiment (Fig. 2). The no measurable coupling constant between H-4 and H-5 was caused by a dihedral angle approximately of 90°. The unambiguously assigned NOE cross-peaks at H-4/H-7, H-5/H-19 and H-5/H-20 were indicative of the same orientation of H-19 and H-5. Besides, the geometry of the double bond between C-6 and C-7 was confirmed as *E*-type based on the NOESY correlation between the signals of H-20/H-8β and H-7/H-8α. A coupling constant of 15.7 Hz between H-9 and H-10 suggested a *E*-configuration of this olefinic bond.

The quantum-mechanically calculated ^{13}C NMR chemical shifts and TDDFT-ECD curve were proved to be powerful and reliable method for assigning the relative and absolute configurations of natural products.[11] The initial Merck Mo-lecular Force Field (MMFF) conformational analysis of the two possible relative orientations structures A (4*S**, 5*R**, 16*S**) and B (4*S**, 5*R**, 16*R**) (Fig. 3) afforded 10 minimum-energy conformers, respectively. The re-optimizations at the B3LYP/6-31G(d) level using polarizable continuum model (PCM) solvent model for CH_3OH produced both 4 conformers above 1% population for structures A and B (Tables S1, S2, Figs. S1 and S2), subsequently, their NMR chemical shifts were calculated at B3LYP/6-311+G(2d,p) level with PCM solvent model for CH_3OH. The experimental chemical shifts for hainanmycin A were compared with the Boltzmann-weighted of the calculated NMR data for each conformers carried out at the B3LYP/6-31G(d) level (Table S3). It is clear that computationally calculated ^{1}H and ^{13}C NMR chemical shifts of structure A displayed a much greater agreement with the experimental data than those of B by it's smaller CMAE (corrected mean absolute error) value (A 1.4 vs B 1.7 of ^{13}C, A 0.09 vs B 0.11 of ^{1}H; Table S3). What'more, the DP4 probability analysis by using *t* distribution and DP4-database 2[12,13] was also identified diastereomer A as the true relative structure with a 99.7% probability (the remaining 0.3% probability was assigned to B).

As for the absolute stereochemistry, the comparison was made between its experimental curve and TDDFT ECD spectra calculated by Boltzmann-weighting of those conformers with PBE0/TZVP (PCM/CH_3OH). As illustrated in Fig. 4, the theoretical mirror-imaged ECD curve of structure A matched very well with the experimental spectrum, thereby defining the absolute configuration of the entire molecule as (4*R*, 5*S*, 16*R*) shown in Fig. 1.

In the general cytotoxicity tested on seven different cell lines (L02, HepG2, SMMC-7721, MCF-7, Hela, CHME-5, PC-3 and HCT-116) with the MTT method as previously reported,[7a] hainanmycin A was inactive. In fact, α,β-unsaturated