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Chemical constituents and chemotaxonomic study on the marine actinomycete *Williamsia* sp. MCCC 1A11233

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ABSTRACT

The marine actinomycete *Williamsia* sp. MCCC 1A11233 was isolated from a deep sea sediment sample of the southwestern Indian Ocean. It was closely related to *Williamsia limnetica* L1505^T with a 16S rRNA gene sequence similarity value of 99.2%. A systematic investigation on its chemical constituents was performed in this study, which led to the isolation of one new (**1**) and 23 known (**2**–**24**) secondary metabolites. By mainly detailed analysis of the 1D and 2D NMR spectroscopic data and comparisons with the published data, the chemical structure of **1** was determined to be 3-benzyl-3α,4β-dihydroxypentan-2-one. Noteworthily, the question on which family *Williamsia* species should belong to was left unsolved at the first beginning when it was found in 1999. Interestingly, the result of this investigation consolidated that *Williamsia* should be placed a chemotaxonomic position in Nocardiaceae Family. Furthermore, 2-aminobenzoic acid derivatives (**8**–**10**) could be taken as chemotaxonomic markers for the genus of *Williamsia*.

1. Subject and source

Strain MCCC 1A11233 was isolated from a sediment sample (−1654 m; E 49.81°, S 37.86°) of the southwestern Indian Ocean in February 2014. Genomic DNA extraction of the isolate was performed as described by Chen and Kuo (1993). The 16S rRNA gene was amplified with universal bacterial primers corresponding to *Escherichia coli* positions 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The 16S rRNA gene sequence of strain MCCC 1A11233 (1372 bp, GenBank accession number KU560459.1) was compared with those available in the GenBank database using the BLAST software program (NCBI) and the EzTaxon-e server (Kim et al., 2012). 16S rRNA gene sequence comparison clearly indicated that strain MCCC 1A11233 was a member of the genus *Williamsia*, which was most closely related to *Williamsia limnetica* L1505^T with a 16S rRNA gene sequence similarity value of 99.2%. A voucher strain of this actinomycete was deposited in the Marine Culture Collection of China (MCCC) under the accession number of MCCC 1A11233.

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2. Previous work

The *Williamsia* species were isolated from diverse environments. Besides the deep-sea sediments, they were also found in land soil, meadow hay, building material, leaf surface, and even human blood. Currently, only nine validly named species were reported, including *Williamsia muralis* (Del Mar Tomas et al., 2005; Kampfer et al., 1999), *Williamsia maris* (Stach et al., 2004), *Williamsia deligens* (Yassin and Hupfer, 2006), *Williamsia marianensis* (Pathom et al., 2006), *Williamsia serinedens* (Yassin et al., 2007), *Williamsia faeni* (Jones et al., 2010), *Williamsia phyllosphaerae* (Kampfer et al., 2011), *Williamsia limnetica* (Sazak and Sahin, 2012), and *Williamsia sterculiae* (Fang et al., 2013). To the best of our knowledge, up to now, there was no report on secondary metabolites of *Williamsia* species.

3. Present study

3.1. Fermentation

The strain *Williamsia* sp. MCCC 1A11233 was cultured on solid-plates using 2216 E medium (Shanghai Yuanye Bio-Technology Co., Ltd.) for 5 d. Then one plate of the strain was inoculated into 5 L Erlenmeyer flask, containing 1.5 L of A3 broth (1.5% starch, 1.5% glycerin, 0.5% potato peptone, 1.5% bacteriological peptone, 3.0% sea salt, and 0.2% $CaCO_3$, pH 7.4). After 48 h of incubation at 28 °C on a rotary shaker at 180 rpm, 1.5 L of seed cultures were transferred to 50 L fermentation cylinder, containing 35 L of A3 broth. And incubation was continued for 10 d at 28 °C, 180 rpm.

3.2. Extraction and isolation

The whole culture medium of two 50 L fermentation cylinder (70 L) was centrifuged to separate the supernatant and mycelia cake. The supernatant was exhaustively extracted three times with ethyl acetate (EtOAc), and the mycelia cake was completely extracted with EtOAc followed by methanol (MeOH). After evaporated in vacuum, the EtOAc extract (19.0 g) and mycelia extract (2.9 g) were then combined on the basis of the result of thin layer chromatography (TLC) analysis to yield a dark brown gum (21.9 g).

The extract was subjected to medium pressure liquid chromatography (MPLC, 36 mm × 310 mm) over ODS using a gradient $H_2O \rightarrow$MeOH (15 mL/min) to yield 12 fractions (Fr.1–Fr.12). Fr.1 (68.0 mg) was separated using Sephadex LH-20 (MeOH) and further purified by preparative TLC using petroleum ether (PE)-EtOAc (5:2) to yield **1** (2.9 mg) and **13** (5.4 mg), respectively. Fr.2 (471.0 mg) was subjected to column chromatography (CC) over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, and 0:1), followed by purification on semi-preparative high performance liquid chromatography (HPLC) at a flow rate of 5.0 mL/min with MeOH–H_2O (20:80) to afford **2** (5.3 mg), **3** (5.2 mg), and **10** (36.9 mg). Fr.3 was separated by CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and silica gel ($CHCl_3$–MeOH, 50:1) to yield **4** (16.0 mg) and **7** (82.0 mg). Similarly, **5** (12.0 mg), **6** (5.3 mg), **24** (4.8 mg), **8** (13.2 mg), and **9** (1.2 mg) were obtained from Fr.4 (90.0 mg), Fr.5 (90.3 mg), Fr.6 (90.0 mg), Fr.8 (547.6 mg), and Fr.9 (35.0 mg), respectively. Fr.10 (875.6 mg) was separated by CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1), followed by CC on silica gel ($CHCl_3$–MeOH, 50:1). Final purified by HPLC at a flow rate of 5.0 mL/min with MeOH–H_2O (30:80) to afford **23** (5.3 mg), **14** (21.7 mg), **15** (51.9 mg), **12** (64.3 mg), **19** (8.5 mg), and **21** (3.2 mg). Using similar procedure, **16** (54.0 mg), **17** (62.0 mg), **18** (37.7 mg), and **11** (18.0 mg) was obtained from Fr.11 (968.4 mg); while **20** (5.9 mg) and **22** (20 mg) were separated from Fr.12 (297.0 mg).

3.3. Structure elucidation

Compound **1** (Fig. 1) was obtained as a colorless amorphous solid with optical rotation $[\alpha]^{D}_{20}$ +3.28 (c 0.25, MeOH). Its molecular formula was determined as $C_{12}H_{16}O_3$ on the basis of the HRESIMS at *m/z* 231.1000 $[M+Na]^+$ (calcd. for $C_{12}H_{16}O_3Na$, 231.0997) and NMR data, accounting for five degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) exhibited one methyl doublet (δ_H 1.11, 3H, d, J = 6.4 Hz, H-10), one methyl singlet (δ_H 1.94, 3H, s), one methylene [δ_H 2.89 (1H, d, J = 13.6 Hz), 2.71 (1H, d, J = 13.6 Hz)], one oxygenated methine (δ_H 3.90, 1H, qui, J = 6.4 Hz), and a monosubstituted benezene (δ_H 7.15–7.22, 5H, m). These signals were supported by resonances in the ^{13}C NMR spectrum at δ_C 17.3 q, 27.3 q, 40.5 t, 70.7 d, 130.1 d (×2), 127.7 d (×2), and 126.1 d (Table 1). In addition, three quaternary carbons were found: one oxygenated sp^3 carbon (84.9 s), one olefinic (136.7 s), and one ketone (212.7 s) groups. Altogether, the NMR data showed very similar to those of 1-phenyl-2,3-butanediol (Awano et al., 1995), except for an additional acetyl group. In the HMBC spectrum, the acetyl methyl (δ_H 1.94) was correlated to δ_C 84.9 s, confirming the connection of the acetyl to C-8 position. In the NOESY spectrum, correlations were found of 8-OH (δ_H 4.64) to H-7a (δ_H 2.71), H_3-10 (δ_H 1.11) and H-2/6 (δ_H 7.17), while H-9 (δ_H 3.90) to H-7b (δ_H 2.89), H_3-12 (δ_H 1.94). This indicated 8-OH/H-7a/H_3-10 were on the same plan, which was opposite to that of H-9/H-7b/H_3-12. By detailed analysis of its ^{1}H–^{1}H COSY, HMBC, and NOESY (Fig. 2), the structure of compound **1** was then assigned undoubtedly as 3-benzyl-3α,4β-dihydroxypentan-2-one.

By comparing the NMR and MS data with literature references, 23 known compounds were determined as nine phenolics: 1-phenyl-2,3-butanediol (**2**) (Awano et al., 1995), 2-phenyl-2,3-butanediol (**3**) (Fristrup et al., 2003), *N*-(4-hydroxyphenethyl) acetamide (**4**) (Peng et al., 2011), 2,4-dichloro-benzoic acid (**5**) (Wright et al., 2005), 2-(4-hydroxyphenyl)ethanol (**6**) (Kwak et al., 2009), *N*-(4-hydroxyphenethyl) acetamide (**7**) (Yu et al., 2014b), 2-aminobenzoic acid (**8**) (Park and Shim, 2014), 2-[(2-