(±)-Meliviticines A and B: Rearranged prenylated acetophenone derivatives from Melicope viticina and their antimicrobial activity

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\textbf{ABSTRACT}

Two new prenylated acetophenone derivatives racemates, meliviticines A (1) and B (2) with unprecedented rearranged skeletons, were isolated from Melicope viticina. Subsequent chiral resolution led to the separation of two pairs of enantiomers, (±)-meliviticines A (1a/1b) and (±)-meliviticines B (2a/2b). Their structures including absolute configurations were elucidated by extensive spectroscopic data, electronic circular dichroism analysis, and X-ray crystallography. A plausible biosynthetic pathway of 1 and 2, involving ring cleavage and rearrangement of the prenylated acetophenone backbone was proposed. All the isolates showed moderate antimicrobial activities with MIC values of 25–50 μg/mL against several bacterial and fungal strains.

1. Introduction

The genus of \textit{Melicope} (Rutaceae) contains about 233 species mainly distributed in the tropical regions of the Eastern Hemisphere [1]. Many species have been used as traditional medicines with diverse pharmacological activities [2–11]. \textit{Melicope} genus is well-known as a rich source of structurally diverse natural products such as alkaloids, flavonoids, benzopyrans, and acetophenones with a wide range of biological activities including antioxidant, antiinflammatory, antiplatelet, analgesic, PTP1B inhibitory, neuraminidase inhibitory, antibacterial, antimalarial, antifungal, and cytotoxic activities [2–18]. Amongst, prenylated acetophenones with skeletal structure of an acetophenone connected with one or more prenyl or geranyl groups, are considered as the most important chemotaxonomic markers of \textit{Melicope} and \textit{Acroanchyta} species [18–22]. Although there have been a large number of prenylated acetophenones isolated [4–6,9–11,14–17,20–22], only a few showed dearomatized ring [11,15,16,18].

\textit{Melicope viticina} (Wallich ex Kurz) T. G. Hartley, a deciduous shrub or tree distributed in Yunnan province, China and Southeast Asia, has never been studied before. To search for more bioactive metabolites from plants, two rearranged nonaromatic prenylated acetophenone derivatives, meliviticines A (1) and B (2) with unprecedented dearomatic skeletons were isolated from the leaves and twigs of \textit{M. viticina} in this study (see Fig. 1). Further chiral-phase separation led to two pairs of enantiomers, (±)-meliviticines A (1a/1b) and (±)-meliviticines B (2a/2b). The antimicrobial activity was evaluated against a panel of bacteria and fungi, and the results showed all the isolates with moderate activities. Herein the isolation, structural elucidation, plausible biosynthetic formation and antimicrobial activity were presented.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured with a Rudolph Autopol I automatic polarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer and CD spectra on an Applied Photophysics Chirascan spectrometer. IR spectra were recorded on a Bruker Tensor 37 infrared spectrometer using KBr disks. NMR spectra were recorded with a Bruker AM-400 instrument. HRESIMS were recorded on a Waters Micromass Q-TOF spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-20 AT and an SPD-M20A PDA detector with a YMC-pack ODS-A column (10 × 250 mm, S-5 μm, 12 nm) and a chiral column (Phenomenex Lux, cellulose-2, 10 × 250 mm, 5 μm). Column
The leaves and twigs of *Melicope viticina* were collected from Xishuangbanna Tropical Botanical Garden, Yunnan Province, China, in August 2017 and identified by Professor You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen has been deposited in School of Pharmaceutical Sciences, Chongqing University (Accession number CRZ2017MV).

### 2.3. Extraction and isolation

The air-dried and powdered leaves and twigs (10.2 kg) of *M. viticina* was extracted with 95% ethanol (3 × 50 L) at room temperature and evaporation of solvent under reduced pressure afforded a crude extract (450 g), which was then suspended in H₂O (1.5 L) and sequentially partitioned with petroleum ether (4 × 1 L), EtOAc (4 × 1 L) and n-BuOH (4 × 1 L). After the evaporation of solvent, the EtOAc fraction (150 g) was chromatographed on a MCI gel column eluted with MeOH-H₂O (3:7–10:0) in gradient to yield five fractions. Fraction 1 (38.9 g) was subjected to a silica gel column eluted with CHCl₃-MeOH (100:1-10:1) to give five fractions F1a–F1e. A portion (1.0 g) of F1c (3.6 g) was fractionated on a silica gel column eluted with petro-EtOAc (3:1-3:1) to get four fractions F1c1–F1c4. F1c1 (110 mg) was separated on an RP-18 CC eluted with MeOH-H₂O (40:60) and then purified by semi-preparative HPLC with MeCN-H₂O (30:70, 3 mL/min) to give 1 (27 mg, tᵣ 12 min). Similarly, F1c2 (180 mg) was separated on an RP-18 CC eluted with MeOH-H₂O (30:70) and then purified by semi-preparative HPLC with MeCN-H₂O (20:80, 3 mL/min) to give 2 (30 mg, tᵣ 12 min). The resolution of racemic mixture 1 and 2 was performed by HPLC equipped with a chiral-phase column (MeCN/H₂O, 35:65 and 30:70, 3 mL/min) to give 1b (8.0 mg, tᵣ 10.3 min) and 1a (9.0 mg, tᵣ 10.8 min) and 2a (10.0 mg, tᵣ 9.0 min) and 2b (11.4 mg, tᵣ 9.5 min), respectively.

### 2.4. Spectroscopic data

Melivitcinines A (1): Colorless amorphous solid; UV (MeOH) λ<sub>max</sub> (log ε) 260 (4.15) nm; IR (KBr) ν<sub>max</sub> 3000–3700 (br), 2976, 1754, 1674, 1618, 1593, 1466, 1379, 1301, 1266, 1240, 1207, 1175, 1040, 942, 847 cm⁻¹; for ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 297.1685 [M+H]<sup>+</sup> (calcd for C₁₄H₂₁O₆, 297.1697); 1a: [α]<sub>D</sub><sup>25</sup> + 48.4 (c 3.33, MeCN); CD (MeCN): Δε (λ₂₃₃) −5.05, (λ₂₂₇) −5.08, (λ₂₁₅) + 5.91; 1b: [α]<sub>D</sub><sup>25</sup> −50.1 (c 1.67, MeCN); CD (MeCN): Δε (λ₂₃₃) + 5.77, (λ₂₂₆) + 5.71, (λ₂₁₅) −5.86.

Melivitcinines B (2): Colorless amorphous solid; UV (MeOH) λ<sub>max</sub> (log ε) 231 (4.05) nm; IR (KBr) ν<sub>max</sub> 3000–3700 (br), 2980, 1793, 1461, 1368, 1227, 1186, 1111, 1037 cm⁻¹; for ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 285.1321 [M+H]<sup>+</sup> (calcd for C₁₃H₂₁O₆, 285.1333); 2a: colorless crystals (petroleum ether/EtOAc, 8:1), mp
142–144°C; [α]D25 +66.4 (c 2.50, MeCN); CD (MeCN): Δε(λ231) +4.25; 2b: [α]D25 −58.0 (c 3.13, MeCN); CD (MeCN): Δε(λ230) −3.08.

2.5. X-ray crystal structure analysis

Single crystal of 2a was collected on an Xcalibur, Onyx, Nova diffractometer equipped with Cu Kα radiation (λ = 1.54184 Å). The structure was determined using direct methods and refined using olex2. All non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms were located by geometrical calculations. The absolute configuration was confirmed by refinement of the Flack parameters. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, CCDC Number 1903989 (2a). These data are available free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for (+) meliviticines B (2a), C14H20O6 (M = 284.30 g/mol): monoclinic, space group C2 (no. 5), a = 23.2522(4) Å, b = 5.84900(10) Å, c = 10.83349(19) Å, β = 99.6403(16)°, V = 1452.57(4) Å3, Z = 4, T = 100 K, μ(Cu Kα) = 0.852 mm−1, Dcalc = 1.300 g/cm3, 13,691 reflections measured (7.714° ≤ 2θ ≤ 144.21°), 2849 unique (Rint = 0.0304, Rsigma = 0.0197) which were used in all calculations. The final R1 was 0.0284 (I > 2σ(I)) and wR2 was 0.0733 (all data). Flack parameter = 0.08 (6).

2.6. Antimicrobial assays

Antimicrobial assays were conducted as described previously [23–25]. All the isolates were evaluated for antibacterial activity against Micrococcus lysodeikticus, Bacillus megaterium, Bacterial pythosom B, Methicillin-resistant Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa strains and antifungal activity against Verticillium dahliae Kleb, Alternaria alternata, Rhiocconia solani, Sclerotinia sclerotiorum, Phytophthora parasitica, Gibberella saubinetii strains.

3. Results and discussion

Meliviticine A (1) possessed a molecular formula of C14H20O6 as determined by the HRESIMS m/z 297.1685 [M+H]+ (calcd for C14H20O6 297.1697), corresponding to 5° of unsaturation (DOUs). The IR spectrum absorption bands at 3397, 1673, and 1617 cm−1 indicated
The presence of hydroxyl, carbonyl, and vinyl groups, respectively. The \( ^1H \) NMR spectrum (Table 1) displayed signals for five tertiary methyl protons (\( \delta \) 1.16, 1.37, 2.38 (each 3H, s), and 1.20 (3H \times 2, s)), one oxymethine (\( \delta \) 4.54 (1H, dd, \( J \) = 10.9, 5.2 Hz)), and six aliphatic protons. The \( ^13C \) NMR spectrum (Table 1) with the help of DEPT experiments and HSQC spectrum resolved 16 carbons into the categories two ketocarbonyl groups (\( \delta \) \( C \) 198.1 and 200.3), one oxygenated double bond (\( \delta \) \( C \) 115.0 and 183.7), five methyls, two sp\(^3\) methylenes, three sp\(^3\) methines (one oxygenated at \( \delta \) \( C \) 94.4), two oxygenated sp\(^3\) quaternary carbons (\( \delta \) \( C \) 72.9 and 69.9), accounting for three of the five DOUs, which suggested that 1 was bicyclic. The planar structure of 1 was elucidated by analysis of the 2D NMR data (Fig. 2). The fragment of oxygenated methine CH-10 and six aliphatic protons CH\(^2\)-CH-CH\(^2\)-CH was established as shown in Fig. 2 by the \( ^1H \)– \( ^1H \) COSY correlations of H-3/H-11, H-2/H-4, H-5/H-9, and H-9/H-10. The HMBC correlations of H-3/C-2, H-4/C-2 and C-6, H-5/C-1 and C-6 together with the \( ^1H \)– \( ^1H \) COSY correlations of H-3/H-4 and H-4/H-5 confirmed the connection of C-2-C-3-C-4-C-5-C-6-C-1. One oxygenated isopropyl group was connected to C-3 by the HMBC correlations from Me-15 and Me-16 to C-3 and one oxygenated sp\(^3\) quaternary carbon C-14 (\( \delta \) \( C \) 72.9). Another oxygenated isopropyl group was connected to C-10 by the HMBC correlations from Me-12 and Me-13 to C-10 and another sp\(^3\) oxygenated quaternary carbon C-14 (\( \delta \) \( C \) 69.9). Besides, one acetyl group was attached to C-1 by the HMBC correlations of Me-8/C-1 and C-7. Unfortunately, two hydroxyl groups could not be assigned due to the absence of their proton resonances when tested in CDCl\(_3\). The connections of C-1 to C-2 and C-6 to C-10 could not be determined either since there were no any useful HMBC correlations observed. To resolve those assignments, the \( ^1H \), \( ^13C \), HSQC and HMBC spectra of 1 were tested again in DMSO-\( d_6 \). Two hydroxyl groups were then attached to C-11 and C-14 as two dimethylcarbinol groups by the HMBC correlations from 11-OH to C-10, C-11, C-12 and C-13, and from 14-OH to C-3, C-14, C-15 and C-16, respectively (Fig. 2). Thus, we could connect the oxygenated methine C-10 (\( \delta \) \( C \) 94.4) to C-6 (\( \delta \) 183.7) belonging to the oxygenated double bond C-6/C-1 through the ether bond to form a typical furanoid ring (ring B), and then the \( \alpha \)-\( \beta \)-unsaturated ketone group was determined to connect C-1 and C-2 to form the ring A as a cyclohexenone moiety by the chemical shift of C-2 (\( \delta \) \( C \) 200.3) and C-7 (\( \delta \) 198.1), which showed the presence of conjugated ketone carbonyl groups. The above analysis was also in accord with the bicyclic structure of 1. Therefore, the gross structure of 1 was established as a nonaromatic prenylated and isopropylated acetophenone derivative.

The relative stereochemistry of 1 was determined by the NOESY experiment (Fig. 3), where the correlations of H-5/H-3 and H-10 suggested they were cofacial, which was also supported by the NOESY correlations of H-2/H-15, H-4a/H-9a, H-4b/H-9b and H-2/H-9/Me-12.

The specific optical rotation value of 1 was almost zero, which

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Table 2

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<th>Strain</th>
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<td>1b</td>
</tr>
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Table 3

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<td>Gibberella saubinetii</td>
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suggested that it could be a racemic mixture. Subsequent chiral HPLC resolution was performed to yield a pair of enantiomers, 1a and 1b, which exhibited opposite optical rotation and electronic circular dichroism (ECD). To elucidate the absolute configurations of them, the theoretical ECD spectra were calculated using the time-dependent density functional theory (TDDFT) at the B3LYP/6-311++G(d,p) level with MeCN as the solvent in the Conductor-like Polarizable Continuum Model (CPCM) [26–28]. The calculated and experimental ECD spectra matched well, which assigned the absolute configurations of 1a and 1b as 3S, 5R, 10R and 3R, 5S, 10S, respectively (Fig. 4).

Melivitine B (2) had the molecular formula of C14H24O4 based on the HRESIMS m/z 285.1321 [M + H]+ (calcd for C14H24O4, 285.1333), corresponding to 5 DOUs. The 1H NMR spectrum (Table 1) displayed signals for four tertiary methyl protons (δH 1.15, 1.32 (each 3H, s), and 1.42 (3H × 2, s)), one oxymethylene (δH 4.26 (1H, dd, J = 10.0, 6.0 Hz), and five aliphatic protons. The 13C NMR together with DEPT and HSQC spectra (Table 1) resolved 14 carbons, including two ester carbonyls (δC 178.4 and 169.3), one double bond (δC 137.4 and 135.3), four methyls, two sp3 methylenes, two sp3 methines (one oxygenated at δC 84.4), two oxygenated sp3 quaternary carbons (δC 84.7 and 70.3), accounting for three of the five DOUs. Therefore 2 was also bicycllic. The planar structure of 2 was established by the 2D NMR spectra (Fig. 2). The fragment of oxygenated methine CH-10 and five aliphatic protons CH2-CH2-CH3 was displayed as established in Fig. 2 by the 1H-1H COSY correlations of H-4/H-5, H-5/H-9 and H-9/H-10. Then the HMBC correlations from H-4, H-5, H-9 and H-10 to C-6 completed the assignment of a five-membered lactone ring (ring B). Comparing with the NMR data of 1, one dimethylcarbinol group was determined and connected to C-10 by the HMBC correlations from Me-12 and Me-13 to C-10 and one sp3 oxygenated quaternary carbon C-11 (δC 70.3). The HMBC correlations of H-4/C-1, C-2 and C-3, and H-5/C-3 assigned the connection of C-1–C-2–C-3–C-4–C-5. One oxygenated isopropyl group was connected to C-3 by the HMBC correlations from Me-15 and Me-16 to C-3 and one oxygenated sp3 quaternary carbon C-14 (δC 84.7). Although the connection of two quaternary carbon C-1 and C-14 could not be determined directly by the 2D NMR spectra, we still temporally assigned the ring B as another five-membered lactone (ring B) by the chemical shift of C-1 (δC 169.3) and C-14. Lastly, one hydroxyl group had to be attached to C-2, which was supported by the chemical shifts of C-2 (δC 137.4) and C-3 (δC 135.3). Thus 2 was established as a rearranged prenylated and isoprenylated acetophenone derivative with aromatic ring cleavage and acetyl group decomposition.

The relative configuration of 2 was determined by analysis of the NOESY spectrum (Fig. 3). The important NOESY correlation of H-10/H-5 suggested that they were cofacial, which was also supported by the NOESY correlations of H-4a/Me-15, H-5/Me-16, H-9/Me-4b and H-9/Me-12.

Similarly, the small specific optical rotation value of 2 showed weak activities with MIC values of 100 μg/mL. The other two fungi, Micrococcus lysodeikticus and Methicillin-resistant Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa, and two fungi, Verticillium dahiae Kleb and Gibberella saubinetii, while for the other two fungi, Rhizoctonia solani and Sclerotinia sclerotiorum, 1a/1b and 2a/2b showed weak activities with MIC values of 100 μg/mL.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103099.

References