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Research Article

Tinctoride A, a New Hopan-Type Triterpenoic Peracid from the Thallus of Lichen *Parmotrema Tinctorum* (Despr. ex Nyl.) Hale

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A new hopan-type triterpenoic peracid, tinctoride A (1), together with three known compounds, zeorin (2), 6β,22-dihydroxyhopane (3), and ergosterol peroxide (4), was isolated from *Parmotrema tinctorum* (Despr. ex Nyl.) Hale. Their chemical structures were identified by extensive 1D and 2D NMR analysis and high-resolution mass spectroscopy and compared with those reported in the literature. The enzyme inhibitory potential of compounds 1–3 against α-glucosidase was investigated, exhibiting nil to weak inhibitory activity.

1. Introduction

Lichens, which are symbiotic organisms having the characteristics of mycobionts and fungi, comprise 17,000 species distributed from tropical to polar regions [1–3]. This unique ability produces various chemical constituents that are found in most of these organisms [4]. Previous chemical investigations of lichens have confirmed the presence of many kinds of aromatic secondary metabolites, such as depsides, depsidones, dibenzofuranes, xanthones, and anthraquinones [5]. Most of these compounds are unique to lichens, but some can also be obtained from fungi and plants [2]. Biological markers have indicated that lichens have potent antioxidant, anticancer, antiarthritic, anti-inflammatory, antihuman immunodeficiency virus (anti-HIV), and antiherpes simplex virus 1 (anti-HSV-1) activities [4, 5].

Parmotrema tinctorum (Despr. ex Nyl.) Hale (Parmeliaceae) (lichenised Ascomycota) is a foliose lichen growing abundantly in various countries, commonly on rocks and trees in moist tropical and temperate areas [6, 7]. The extract of *P. tinctorum* exhibits significant α-glucosidase, α-amylase, and aldose reductase inhibition [7], and some isolated

compounds manifest moderate in advanced glycation end product formation inhibition activities [8]. However, only a few publications have reported the chemical investigation of *P. tinctorum*, resulting in the isolation of some monoaromatic and aromatic compounds [8–12].

The present paper reports the results of the isolation and elucidation of a new triterpenoid (1) and three known compounds (2-4) from *P. tinctorum*. Moreover, the enzyme inhibitory activity of compounds 1-3 against α-glucosidase is described.

2. Materials and Methods

2.1. General Experimental Procedure. Column chromatography (CC) was performed on 60–120-mesh silica gel. The IR spectra were measured on Frontier FTIR/NIR spectrometers (Perkin Elmer, USA). NMR spectra (1D and 2D) were recorded on a Bruker Avance spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. HRESIMS were recorded on a Bruker MicrOTOF-Q II mass spectrometer. Thin-layer chromatography was carried out on precoated Kieselgel 60 F_{254} or silica gel 60 RP-18 $F_{254}S$ (Merck). Spots were visualized by spraying with 5% vanillin in acidic aqueous solution, followed by heating.

2.2. Plant Material. The thalli of lichen *P. tinctorum* were collected in the Lam Dong province of Vietnam in May 2020. The scientific name of the plant was identified by Dr. Vo Thi Phi Giao, Faculty of Biology-Biotechnology, University of Science, National University, Ho Chi Minh City, Vietnam. A voucher specimen PHH0011338 was deposited in the Herbarium of the University of Science, Ho Chi Minh City Vietnam National University (PHH).

2.3. Isolation and Purification. The air-dried roots (2.5 kg) were extracted exhaustively with ethyl acetate (10 L × 3) and methanol (10 L × 3) at room temperature. The filtered solution was evaporated under reduced pressure to afford two crudes: E (105.2 g) and M (135.5 g). Crude E was washed with ethyl acetate to yield the liquid EL (23.5 g) and precipitate EP (75.8 g). EL was applied to Sephadex LH-20 and eluted with methanol to obtain EL1–3, and EL2 was then fractionated using C-18 silica gel CC with a gradient system of water-methanol (1 : 8), affording five fractions (EL2A–E). Fraction EL2D (16.6 g) was selected for further fractionation by silica gel CC, using a solvent system consisting of n-hexane/$CHCl_3$/ethyl acetate/acetone (4 : 0.2 : 0.2 : 0.2) to afford fractions EL2D1–D5. Subfraction EL2D3 (1.43 g) was rechromatographed by silica gel CC using an n-hexane/$CHCl_3$/ethyl acetate/acetone (5 : 0.2 : 0.2 : 0.2) solvent system, affording compounds 1 (1.6 mg) and 4 (25.0 mg). Fraction EL2D1 (0.63 g) was rechromatographed to afford compounds 2 (3.2 mg) and 3 (5.2 mg), by the same procedure used for EL2D3.

2.3.1. Tinctoride. A (1). Colorless gum; IR (KBr, cm^{-1}): 3564, 2925, 2849, 1739, 1709, 1459, 1374, 1247, 1032; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 4.66 (1H, dd, J = 4.8, 11.6 Hz, H-1), 4.58 (1H, dd, J = 4.4, 12.4 Hz, H-3), 2.36 (1H, m, H-21), 2.33 (1H, m, H-22), 2.02 (3H, s, H-34), 1.97 (3H, s, H-32), 1.89 (1H, m, H-2a), 1.86 (1H, m, H-20a), 1.66 (1H, m, H-2b), 1.62 (1H, m, H-20a), 1.54 (2H, m, H-6), 1.52 (1H, m, H-19a), 1.48 (2H, m, H-16), 1.47 (2H, m, H-9, H-12a), 1.45 (1H, m, H-11a), 1.43 (1H, m, H-20a), 1.40 (1H, m, H-7a), 1.32 (2H, m, H-12b, H-13), 1.31 (2H, m, H-15), 1.30 (1H, m, H-11b), 1.23 (1H, m, H-17), 1.20 (1H, m, H-7b), 1.13 (3H, d, J = 6.4 Hz, H-29), 1.00 (3H, s, H-25), 0.94 (3H, s, H-26), 0.90 (3H, s, H-27), 0.89 (1H, m, H-19b), 0.83 (6H, s, H-23, H-24), 0.75 (1H, d, J = 2.8, 11.2 Hz, H-5), 0.70 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 182.0 (C-30), 170.5 (C-33), 170.3 (C-31), 80.5 (C-1), 76.5 (C-3), 53.8 (C-17), 52.9 (C-5), 50.8 (C-9), 48.7 (C-13), 44.4 (C-18), 42.7 (C-21), 42.3 (C-22), 42.3 (C-8), 42.1 (C-14), 42.1 (C-10), 41.0 (C-19), 37.9 (C-4), 33.5 (C-15), 33.1 (C-7), 30.1 (C-2), 28.0 (C-23), 26.7 (C-20), 23.9 (C-12), 23.0 (C-11), 21.9 (C-34), 21.2 (C-32), 19.9 (C-16), 17.9 (C-6), 17.8 (C-29), 17.0 (C-26), 16.7 (C-27), 16.2 (C-24), 15.8 (C-28), 13.0 (C-25); HRESIMS m/z: $[M + Na]^{+597.3794}$ for $C_{34}H_{54}O_7Na$ (calcd. 597.3767).

2.4. α-Glucosidase Activity Test. The α-glucosidase inhibitory activity of the compounds was investigated using a method from Wan et al. [13]. All samples were examined in triplicate at different concentrations to obtain the IC_{50} value of each compound. The mean values and standard deviations were retained. Briefly, 120 μL of extract were preincubated with 20 μL of α-glucosidase (1 unit/mL) in 0.1 M potassium phosphate buffer (pH 6.8) at 37°C for 15 min. The reaction was then initiated by adding 20 μL of 5 mM para-nitrophenyl-α-D-glucopyranoside (PNPG) in 0.1 M potassium phosphate buffer, and the mixture was further incubated for 15 min. The reaction was terminated by the addition of 80 μL of 0.2 M Na_2CO_3 in 0.1 M potassium phosphate buffer, and the absorbance of the mixture was recorded at 405 nm. The results were expressed as % inhibition of enzyme activity and calculated according to the following equation:

$$\text{Inhibition}\,(\%) = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}}\right)\right] \times 100. \quad (1)$$

3. Results and Discussion

3.1. Structural Elucidation. The ethyl acetate extract of *P. tinctorum* was separated by CC over silica gel normal phase and reversed-phase RP C-18, and sephadex LH-20 to afford a new triterpene, tinctoride A (1), and three known compounds, zeorin (2) [14], 6β,22-dihydroxyhopane (3) [15], and ergosterol peroxide (4) [16] (Figure 1).

Compound 1 was purified as a colorless gum with molecular formula $C_{34}H_{54}O_7$, as assumed by the sodiated molecular ion peak at m/z 597.3794 (calcd. for $C_{34}H_{54}O_7$ + Na, 597.3767) on the HRESI mass spectrum. The ^{13}C NMR and HSQC spectra of 1 revealed 34 carbon signals, including 9 methyl, 9 methylene, 8 methine, and 8 quaternary carbons. In addition, the ^{1}H NMR spectrum showed six singlets (δ_H 1.00, 0.94, 0.90, 0.83 × 2, 0.70) and one doublet (δ_H 1.13, d, 6.4), and the key HMBC correlations were observed from six methyl groups, H_3-23 (δ_H 0.83), H_3-24 (δ_H 0.83), H_3-25 (δ_H 1.00), H_3-26 (δ_H 0.94),

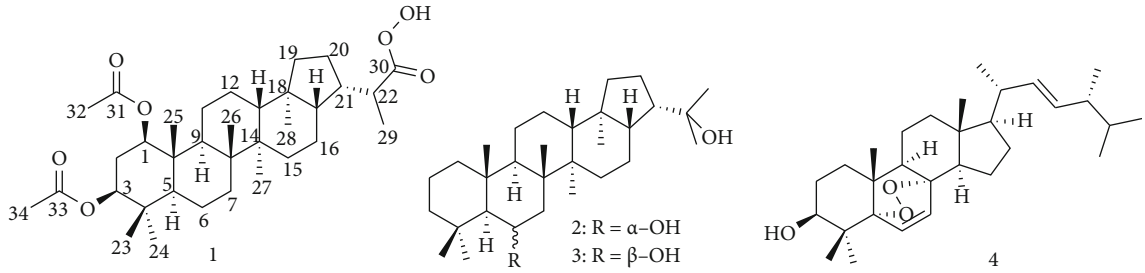

Figure 1: Chemical structure of compounds 1-4.

H_3-27 (δ_H 0.90), and H_3-28 (δ_H 0.70), to their attached quaternary carbons C-4 (δ_C 37.9), C-4, C-10, C-8 (δ_C 42.3), C-14 (δ_C 42.1), and C-18 (δ_C 44.4), respectively, suggesting that 1 was a hopan-type triterpenoid [17], related to the known cometabolites 2, 3. In addition, the HMBC correlations from the methyl protons at δ_H 2.02 (H_3-34) to C-33 (δ_C 170.5) and δ_H 1.97 (H_3-32) to C-31 (δ_C 170.3) suggested the occurrence of two acetoxycarbonyl groups located at C-1 and C-3, respectively, which was further backed up by the HMBC crosspeak of the oxymethine proton H-1 (δ_H 4.66) to C-31 and H-3 (δ_H 4.58) to C-33. Furthermore, the key HMBC of the doublet methyl H_3-29 (δ_H 1.13, d, $J = 6.4$ Hz) to C-21 (δ_C 42.7), C-22 (δ_C 42.3), and C-30 (δ_C 182.0) indicated that a methyl group belonging to the isopropyl moiety located at C-21 (ring E) was oxidated to a carboxyl function (Supplementary material Figure S1). The FTIR spectrum of compound 1 also revealed the presence of free OH, OH stretching, C=O stretching, and OH bending vibrations of the peracid functional group at 3564, 3293, 1739, and 1459 cm^{-1}, respectively [18], indicating a peracid moiety located at C-30, which was further clearly evidenced by the value of its chemical shift at δ_C 182.0, instead of approximately 184 ppm in the case of an acid function [15], and also by the molecular formula $C_{34}H_{54}O_7$ from the HRESI mass spectrum. In addition, the chromatography experiment between 1 and the crude fraction revealed that 1 was a minor substance in *P. tinctorum* (Supplementary material Figure S2).

As to the relative stereochemistry of C-1 and C-3, the elevated coupling constant of H-1 ($J_{H-1,H-2} = 11.6$, 4.8 Hz) and H-3 ($J_{H-2,H-3} = 12.4$, 4.4 Hz) determined the axial position of both of these oxymethine protons. Furthermore, the syn-orientation (β-orientation) of H-1 (δ_H 4.66), H-3 (δ_H 4.58), and H-5 (δ_H 0.75) was definitely indicated by pair-to-pair NOESY interactions (Supplementary material Figure S1). From all the above data, compound 1, namely, tinctoride A, was readily elucidated as 1β,3β-diacetoxy-21α-hopan-29-oic peracid.

3.2. α-Glucosidase Inhibition Assay. The enzyme inhibition of compounds 1-3 against α-glucosidase was evaluated (Supplementary material Table S1). Triterpenoids 1–3 exhibited weak (compound 2, IC_{50} 258.87 μM) or no (compounds 1 and 3) α-glucosidase inhibitory activity compared with acarbose (positive control, IC_{50} 108.08 μM). Among these compounds, 2 showed the highest α-glucosidase inhibitory activity, similar to that reported in the previous study [19], while the 6β-OH orientation of compound 3 led to a decrease in its α-glucosidase inhibitory activity, in spite of its molecular formula being closely related to compound 2. It is worth noting that the stereochemistry of C-6 hydroxyl substitutions in hopan-type triterpenoids could play an important role in their α-glucosidase inhibitory activities. The extract of *P. tinctorum*, however, displayed significant α-glucosidase inhibition [7], which indicates that the hopan-type triterpenoids are not the most potent α-glucosidase inhibitory component in the ethyl acetate fraction of *P. tinctorum*. Due to the reports of various phenolic compounds [6, 8, 9, 11, 12], further isolation of other compounds from the ethyl acetate fraction, such as phenolic compounds or other types of triterpenoids, will be important research for the discovery of active compounds against α-glucosidase.

4. Conclusions

Three hopan-type terpenoids, including one new peracid, tinctoride A, and two known compounds, zeorin (2) and 6β,22-dihydroxyhopane (3), together with ergosterol peroxide (4), were isolated from the lichen *P. tinctorum*. Their chemical structures were identified by extensive 1D and 2D NMR analysis and high-resolution mass spectroscopy and compared with those reported in the literature. To the best of our knowledge, compound 1 is a new peracid, while compounds 2–4 have not previously been isolated from *P. tinctorum*. Compounds 1-3 displayed nil or weak inhibitory activity against α-glucosidase.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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Supplementary Materials

Supplementary material relating to this article is available online, alongside Table S1 and Figures S1–S10. *(Supplementary Materials)*

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