NATURAL PRODUCT RESEARCH
2022, VOL. 36, NO. 8, 2009–2014
https://doi.org/10.1080/14786419.2020.1839463

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Three new phenolic compounds from the lichen *Ramalina peruviana* Ach. (Ramalinaceae)

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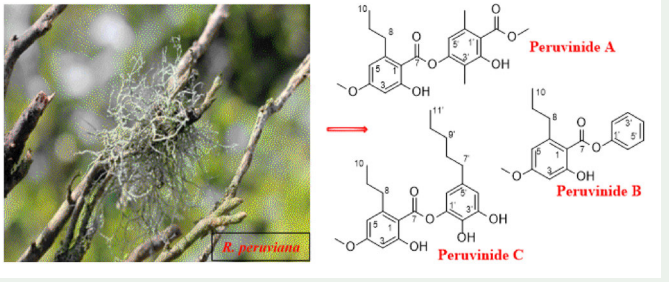
ABSTRACT

Three new phenolic compounds, peruvinides A-C were isolated from the lichen *Ramalina peruviana* Ach. (Ramalinaceae). Their structures were unambiguously determined by extensive spectroscopic analyses and comparison with literature data. Peruvinides A and B bearing unusual moieties were found for the first time among lichen metabolites.



ARTICLE HISTORY
Received 10 September 2020
Accepted 15 October 2020

KEYWORDS
Ramalina peruviana Ach; Ramalinaceae; lichen; depside; peruvinides A–C

1. Introduction

Lichens have been considered as valuable sources of phytochemical ingredients, producing various metabolites, for examples depsides, depsidones, and diphenyl ethers (Koparal et al. 2006; Ly et al. 2015; Nguyen et al. 2018). Lichen metabolites exhibited a wide range of biological activities such as antimicrobial, analgesic and antipyretic (Ingolfsdottir 2002), anti-inflammatory (Cordeiro et al. 2008), antitumor and antimutagenic (Honda and Vilegas 1999; Boustie and Grube 2005; Einarsdóttir et al. 2010; Bačkorová et al. 2011), enzyme inhibitory activities (Boustie and Grube 2005). The

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1839463.

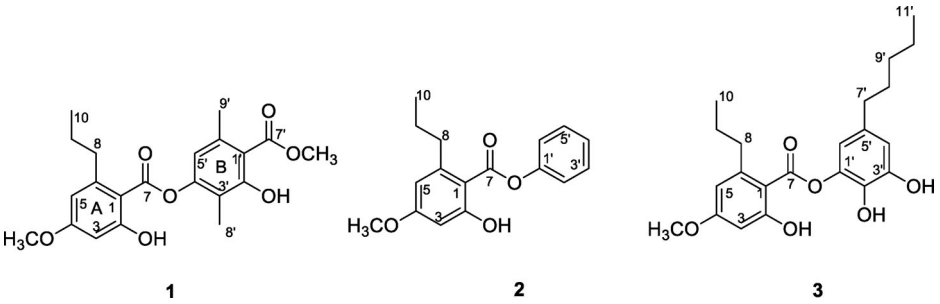

Figure 1. The chemical structures of **1**–**3**.

Ramalina genus has a widespread distribution with the occurrence of over 240 species (Kirk et al. 2008). Phytochemical data of *Ramalina peruviana* Ach., a native lichen in the South of Vietnam are little with two reports having identified several polysaccharides and depsides without isolation (Cordeiro et al. 2003, 2004). In the course of our systematic research on Vietnamese lichens (Duong et al. 2015, 2017, 2018, 2020), we have examined *R. peruviana*. In this paper, we reported the isolation and structural elucidation of three new compounds, peruvinides A-C (Figure 1). Their chemical structures were elucidated by interpretation of spectroscopic data.

2. Results and discussion

Compound **1**, a white amorphous powder, had the molecular formula as $C_{21}H_{24}O_7$ on the basis of the HRESIMS data with a sodiated ion peak at *m/z* 411.1417 (calcd. for $C_{21}H_{24}O_7$+Na, 411.1420). The ^{1}H-NMR spectrum of **1** showed the presence of two hydrogen-bond hydroxy groups [δ_H 11.93 (1H, s, 2′-OH) and 11.47 (1H, s, 2-OH)], three aromatic protons [δ_H 6.50 (1H, s, H-5′) and 6.39 (2H, s, H-3 and H-5)], two methoxy groups [δ_H 3.98 (3H, s, 7′-OCH_3) and 3.84 (3H, s, 4-OCH_3)], two methylene groups [δ_H 2.97 (2H, t, $J = 7.5$ Hz, H_2-8) and 1.70 (2H, m, H_2-9)], and three methyl groups [δ_H 2.54 (3H, s, H_3-9′), 2.09 (3H, s, H_3-8′), and 0.95 (3H, t, $J = 7.5$ Hz, H_3-10]. The ^{13}C-NMR spectrum displayed the presence of 21 carbons including two carbonyl ester carbons at δ_C 172.4 (C-7′) and 169.8 (C-7), two methoxy carbons at δ_C 55.6 (4-OCH_3) and 52.4 (7′-OCH_3), two methylenes at δ_C 39.2 (C-8) and 25.4 (C-9), three methyls at δ_C 24.2 (C-9′), 14.4 (C-10), and 9.5 (C-8′), and 12 aromatic carbons in the range of 99–167 ppm, in which four were oxygenated (Table S1).

The ^{1}H–^{1}H COSY correlations showed the spin system through H_2-8–H_2-9–H_3-10, indicating the presence of the *n*-propyl chain (Figure 1). The HMBC correlations of the methylene protons at δ_H 2.97 (H_2-8) to carbons at δ_C 103.7 (C-1), 111.5 (C-5), 148.4 (C-6), 25.4 (C-9), 14.4 (C-10) suggested that the *n*-propyl group was attached to the A-ring at C-6. HMBC correlations of 2-OH (δ_H 11.47) to C-1, C-2 (δ_C 166.8), and C-3 (δ_C 99.2), of all H-3 (δ_H 6.39), H-5 (δ_H 6.39), and 4-OCH_3 (δ_H 3.84) to C-4 (δ_C 165.0) defined the positions of 2-OH and 4-OCH_3. The chemical structure of the A-ring was reminiscent to those of divaricatinic acid (Lai et al. 2013) (Figure S2).

In the so-called B-ring, HMBC cross peaks of 2′-OH (δ_H 11.93) to carbons C-1′ (δ_C 110.2), C-2′ (δ_C 163.0), and C-3′ (δ_C 117.2) and of H_3-8′ (δ_H 2.09) to C-2′, C-3′, and C-4′ (δ_C 152.6) defined the neighboring positions of these groups. In addition, HMBC

correlations of H_3-9′ (δ_H 2.54) to C-1′, C-5′ (δ_C 116.2), and C-6′ (δ_C 139.9) and of H-5′ (δ_H 6.50) to C-1′, C-3′, and C-4′ indicated the connectivity as arising through C-1-C-6. HMBC correlations of H-5′ and 7′-OCH_3 (δ_H 3.98) to C-7′ (δ_C 172.4) indicated the presence of the methyl ester group at C-1′, finalizing the chemical structure of the B-ring (Figure 1). This chemical feature was highly similar to those of methyl β-orsellinate (Duong et al. 2015). The depside linkage between A-ring and B-ring was defined by comparison of the chemical shift of C-4′ (δ_C 152.6) with those of lichen depsides (Huneck and Yoshimura 1996) and HRMS data. Combined, the chemical structure of **1** was determined as shown, namely peruvinide A. The chemical structure of **1** was highly similar to 2′-*O*-methylnorobusatic (Elix et al. 1990), proposing that they shared the same biosynthesis. The only difference is the presence of the *n*-propyl group in A-ring of **1** instead of the methyl group. Compound **1** represented the novel skeleton among lichen depsides.

Compound **2** was isolated as a white amorphous powder. The molecular formula was determined by HR-ESI-MS through the protonated ion peak at *m/z* 287.1299 (calcd. for $C_{17}H_{18}O_4$+H, 285.1284). Detailed comparison of the 1 D NMR data of compounds **2** and **1** (Table S1) indicated that they shared the same A-ring structure. The difference is the replacement of the B-ring in **1** by the phenyl group in **2**. Consequently, compound **2** was elucidated as shown, namely peruvinide B. It is worth noting that the presence of the phenyl ester group in **2** is unusual among lichen metabolites.

Compound **3**, a white amorphous powder, had the molecular formula of $C_{22}H_{28}O_6$ through the deprotonated ion peak at *m/z* 387.1816 in HRESIMS data (calcd. for $C_{22}H_{28}O_6$−H, 387.1808). The careful comparison of NMR data of **1** − **3** (Table S1) indicated that **3** shared the same A-ring as **1** and **2**. The differences are in the structural changes of B-ring. At first, the presence of the *n*-pentyl group was confirmed by the COSY experiment through H_2-7′−H_2-8′−H_2-9′−H_2-10′−H_3-11′. Next, two aromatic protons at δ_H 6.73 (H-6′) and 6.52 (H-1′) showed the HMBC correlations to C-7′ and C-5′ while H_2-7′ showed HMBC cross peaks to C-1′, C-5′, and C-6′, confirming the position of the *n*-pentyl group. The other positions of the B-ring were oxygenated due to the downfield ^{13}C chemical shifts of C-2′, C-3′, and C-4′. This chemical feature was found previously in sekikaic acid derivatives isolated from the *Ramalina* species, i.e., *R. farinacea* (Lai et al. 2013; Ly et al. 2015). Taken together, the chemical structure of **3** was elucidated as shown, namely peruvinide C.

3. Experimental

3.1. General experimental procedures

NMR spectra (1 D and 2 D) were recorded on Bruker Avance 500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) using residual solvent signals as internal references: chloroform-*d* at δ_H 7.26 and δ_C 77.16. HRESIMS were recorded on a Bruker micrOTOF-Q II using the positive mode for compounds **1** and **2** and the negative mode for compound **3**. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F254 or silica gel 60 RP–18 F254S (Merck) and spots were visualized by spraying with

10% H_2SO_4 solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

3.2. Plant material

The thalli of *Ramalina peruviana* Ach. (Ramalinaceae) were collected at Cau Dat farm, Da Lat city, Lam Dong province, Vietnam in December 2017. The scientific name of the lichen was authenticated by Dr. Vo Thi Phi Giao, Faculty of Biology, University of Science, National University – Ho Chi Minh city. A voucher specimen (No US–B070) was deposited in the Herbarium of Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University - Ho Chi Minh City.

3.3. Extraction and isolation

The lichen thalli were washed, dried, ground into powder (1.3 kg) and was exhaustedly extracted by acetone at ambient temperature using the previously reported method (Devi et al. 2020; Phan et al. 2020). After filtrated, the acetone solution was evaporated at the reduced pressure to yield the crude acetone extract (69.1 g). This crude (69.1 g) was subjected to silica gel solid phase extraction and eluted consecutively with *n*-hexane, chloroform, acetone, and methanol to afford four extracts: *n*-hexane (3.22 g), chloroform (49.77 g), acetone (11.78 g), and methanol (3.57 g), respectively (Huynh et al. 2016).

The chloroform extract (49.77 g) was applied to silica gel column chromatography, eluted with a gradient of *n*-hexane–chloroform (10:0–0:10, v/v) to give 10 fractions, C1–C10. Fraction C7 (1.29 g) was silica gel rechromatographed, eluted in the same manner as previously mentioned to obtain three compounds **1** (2.5 mg), **2** (2.0 mg), and **3** (2.3 mg).

3.3.1. Peruvinide A (1)

White amorphous powder; HRESIMS *m/z* 411.1417 ($[M + Na]^+$ calcd. For $C_{21}H_{24}O_7$+Na, 411.1420); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 11.93 (1H, s, 2′-OH), 11.47 (1H, s, 2-OH), 6.50 (1H, s, H-5′), 6.39 (2H, s, H-3 and H-5), 3.98 (3H, s, 7′-OCH_3), 3.84 (3H, s, 4-OCH_3), 2.97 (2H, t, $J = 7.5$ Hz, H-8), 2.54 (3H, s, H-9′), 2.09 (3H, s, H-8′), 1.70 (2H, m, H-9), 0.95 (3H, t, $J = 7.5$ Hz, H-10); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 172.4 (C-7′), 169.8 (C-7), 166.8 (C-2), 165.0 (C-4), 163.0 (C-2′), 152.6 (C-4′), 148.4 (C-6), 139.9 (C-6′), 117.2 (C-3′), 116.2 (C-5′), 111.5 (C-5), 110.2 (C-1′), 103.7 (C-1), 99.2 (C-3), 55.6 (4-O**C**H_3), 52.4 (7′-O**C**H_3), 39.2 (C-8), 25.4 (C-9), 24.2 (C-9′), 14.4 (C-10), 9.5 (C-8′).

3.3.2. Peruvinide B (2)

White amorphous powder; HRESIMS *m/z* 287.1299 ($[M + H]^+$ calcd. For $C_{17}H_{19}O_4$, 2857.1284). ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 11.48 (1H, *s*, 2-OH), 7.46 (2H, d, $J = 7.5$ Hz H-3′ and H-5′), 7.31 (1H, t, $J = 7.5$ Hz, H-4′), 7.19 (2H, t, $J = 8.0$ Hz, H-2′ and H-6′), 6.39 (1H, d, $J = 2.5$ Hz, H-5), 6.38 (1H, d, $J = 2.5$ Hz, H-3), 3.84 (3H, s, 4-OCH_3), 2.99 (2H, t, $J = 7.5$ Hz, H-8), 1.71 (2H, m, H-9), 0.96 (3H, t, $J = 7.5$ Hz, H-10); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 170.5 (C-7), 166.6 (C-2), 164.8 (C-4), 150.1 (C-1′), 148.3 (C-6), 129.8 (C-3′), 129.8 (C-5′), 126.4 (C-4′), 121.8 (C-2′), 121.8 (C-6′), 111.5 (C-5), 104.1 (C-1), 99.1 (C-3), 55.5 (4-O**C**H_3), 39.3 (C-8), 25.4 (C-9), 14.4 (C-10).

3.3.3. Peruvinide C (3)

White amorphous powder; HRESIMS *m/z* 387.1816 ([M–H]$^-$ calcd. for $C_{22}H_{28}O_6$-H, 387.1808); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 11.25 (1H, s, 2-OH), 6.73 (1H, s, H-6′), 6.51 (1H, s, H-4′), 6.39 (2H, s, H-3 and H-5), 3.85 (3H, s, 4-OCH_3), 2.98 (2H, t, $J = 7.5$ Hz, H-8), 2.52 (2H, t, $J = 7.5$ Hz, H-7′), 1.71 (4H, m, H-9 and H-9′), 1.32 (4H, m, H-8′ and H-10′), 0.98 (3H, t, $J = 7.0$ Hz, H-10), 0.89 (3H, t, $J = 6.5$ Hz, H-11′); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 170.1 (C-7), 166.8 (C-2), 165.3 (C-4), 148.7 (C-6), 146.8 (C-1′), 139.0 (C-3′), 137.0 (C-5′), 132.8 (C-2′), 113.5 (C-6′), 113.2 (C-4′), 111.9 (C-5), 103.6 (C-1), 99.2 (C-3), 55.6 (4-O**C**H_3), 39.3 (C-8), 35.4 (C-7′), 31.5 (C-8′), 31.0 (C-9′), 25.3 (C-9), 22.7 (C-10′), 14.4 (C-10), 14.2 (C-11′).

Acknowledgments

The authors would like to thank Dr. Thi-Phi-Giao Vo, Department of Biology, University of Science, University of Science, National University HCM City, Vietnam for the authentification of the scientific name for the studied species.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104-NN.01-2019.45.

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