NATURAL PRODUCT RESEARCH
2022, VOL. 36, NO. 15, 3945–3950
https://doi.org/10.1080/14786419.2021.1901288

Usneaceratins A and B, two new secondary metabolites from the lichen *Usnea ceratina*

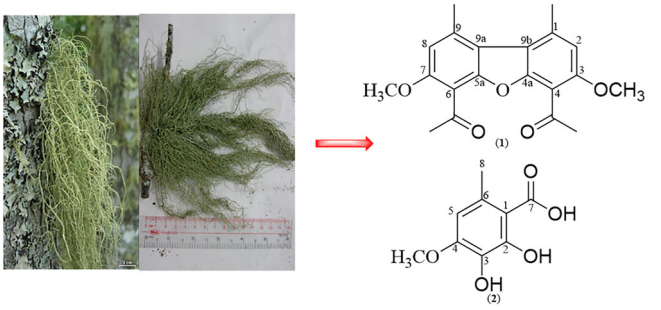
Van Muoi Bui[a], Bui Linh Chi Huynh[b], Nguyen Kim Tuyen Pham[c], Thi Anh Tuyet Nguyen[d], Thi Thuy Trang Nguyen[e], Kim Phi Phung Nguyen[a] and Tan Phat Nguyen[f,g]

[a]Department of Chemistry, University of Science, National University, Ho Chi Minh City, Vietnam; [b]Department of Science, Dong Nai University, Bien Hoa City, Vietnam; [c]Faculty of Environmental Science, Sai Gon University, Ho Chi Minh City, Vietnam; [d]Department of Chemistry, University of Education, Ho Chi Minh City, Vietnam; [e]Phong Phu High School, Ho Chi Minh City, Vietnam; [f]Faculty of Chemistry, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [g]Bioactive Compounds Laboratory, Institute of Chemical Technology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

ABSTRACT

Two new compounds, comprising one dibenzofuran, named usneaceratin A (**1**), and one phenolic acid, named usneaceratin B (**2**), together with one known dibenzofuran, isousnic acid (**3**), and two known phenolics, orsellinic acid (**4**) and methyl orsellinate (**5**) were clarified from the lichen *Usnea ceratina* using variously chromatographic methods. Their structures were testified by comprehensive HR-ESI-MS, and NMR spectroscopic analysis, and comparison with published data. Their α-glucosidase inhibitory activity of all compounds was measured. Usneaceratin B (**2**) possessed better inhibition against α-glucosidase enzyme (IC_{50} value of 41.8 μM) than the standard drug acarbose (IC_{50} value of 214.50 μM).

Graphical Abstract



ARTICLE HISTORY
Received 28 December 2020
Accepted 5 March 2021

KEYWORDS
Usnea ceratina; usneaceratin; dibenzofuran; phenolic; α-glucosidase inhibitory activity

*CONTACT Huynh Bui Linh Chi hainhanchi@yahoo.com.vn; Nguyen Tan Phat ntphat@ict.vast.vn

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2021.1901288.

1. Introduction

Lichens, symbiotic products of a mycobiont and photobiont, are known to produce a range of secondary compounds, such as depsides, depsidones and diphenyl ethers, some of which have antibiotic, antitumor and anticancer activities (Ahmadjian and Hale 1973; Huneck and Yoshimura 1996; Nash 2008). However, lichens remain a relatively unexplored source of biologically active compounds as compared with higher plants.

The *Usnea* genus belonging to the family Parmeliaceae, comprised more than 360 species is one of the mostly pale grey-green fruticose lichens (Prateeksha et al. 2016). Phytochemical investigations of *Usnea* species declared phenolics, depsides and depsidones as the main ingredients, furthermore, benzofurans, terpenoids and steroids were verified (Prateeksha et al. 2016). In our earlier article, five depsidones were reported (Bui et al. 2020). As part of our continuing study on α-glucosidase inhibitors (Nguyen et al. 2015; Nguyen, Le, Phan, Bui, Mai, 2016, Nguyen, Le, Phan, Bui, Pham, et al. 2016) as well as bioactive composition of lichens (Huynh et al., 2016, 2020, 2021), this article reports the separation, structural indication and α-glucosidase inhibition of two new compounds and three known ones (**1–5**) from *Usnea ceratina* collected on from the bark of various trees at Paksong town, Paksong district, Champasack province, Laos.

2. Results and discussion

The ethyl acetate extract of the lichen *U. ceratina* was applied to column chromatography over silica gel normal-phase to afford one dibenzofuran, usneaceratin A (4,6-diacetyl-3,7-dimethoxy-1,9-dimethyldibenzofuran) (**1**), one phenolic acid, usneaceratin B (3-hydroxy-4-*O*-methylorsellinic acid) (**2**), together with three known compounds, isousnic acid (**3**), orsellinic acid (**4**) and methyl orsellinate (**5**) (Figure 1).

Compound **1** was isolated as a white solid. Its molecular formula was established as $C_{20}H_{20}O_5$ by HR-ESI-MS data ($[M-H]^-$ *m/z* 339.1252, calcd. for $C_{20}H_{19}O_5$, 339.1227). The ^{1}H-NMR spectrum of **1** (Table S1) revealed two aromatic protons at δ_H 7.06 (2H, *s*, H-2, H-8), two methoxy signals at δ_H 3.95 (6H, *s*, 3-OC$\underline{H}_3$, 7-OC$\underline{H}_3$), four methyl groups at δ_H 2.47 (6H, *s*, 1-C$\underline{H}_3$, 9-C$\underline{H}_3$) and 2.43 (6H, *s*, 4-COC$\underline{H}_3$, 6-C$\underline{O}_3$). The combination of ^{13}C-NMR and HSQC spectra of **1** (Table S1) exposed 20 carbons including two carbonyl carbons at δ_C 199.3 (4-$\underline{C}$OCH$_3$, 6-$\underline{C}$OCH$_3$), four oxygenated aromatic carbons at δ_C 168.5 (C-4a, C-5a), and 161.7 (C-3, C-7), six quaternary aromatic carbons at δ_C 146.5 (C-1, C-9), 132.8 (C-4, C-6), and 113.6 (C-9a, C-9b), two methine aromatic carbons at δ_C 112.2 (C-2, C-8), two methoxy carbons at δ_C 56.7 (3-O$\underline{C}$H$_3$, 7-O$\underline{C}$H$_3$), and four methyl carbons at δ_C 21.1 (1-$\underline{C}$H$_3$, 9-$\underline{C}$H$_3$) and 26.9 (4-COC$\underline{H}_3$, 6-COC$\underline{H}_3$). The ^{13}C & ^{1}H-NMR data of **1** (Table S1) affirmed a symmetrical dibenzofuran skeleton similar 3,7-dihydroxy-1,9-dimethyldibenzofuran (Tanahashi et al. 2001), except for the presence of two acetyl functions, according to two carbonyl carbons at δ_C 199.3 (4-$\underline{C}$OCH$_3$, 6-$\underline{C}$OCH$_3$), two methyl carbons at δ_C 26.9 (4-COC$\underline{H}_3$, 6-COC$\underline{H}_3$) correlated with methyl protons at δ_H 2.43 (4-COC$\underline{H}_3$, 6-COC$\underline{H}_3$), and two methoxy carbons at δ_C 56.7 (3-O$\underline{C}$H$_3$, 7-O$\underline{C}$H$_3$) corresponded with protons at δ_H 3.95 (3-OC$\underline{H}_3$, 7-OC$\underline{H}_3$), and two quaternary aromatic carbons at δ_C 132.8 (C-4, C-6) in **1**, and the absence of two methine aromatic carbons

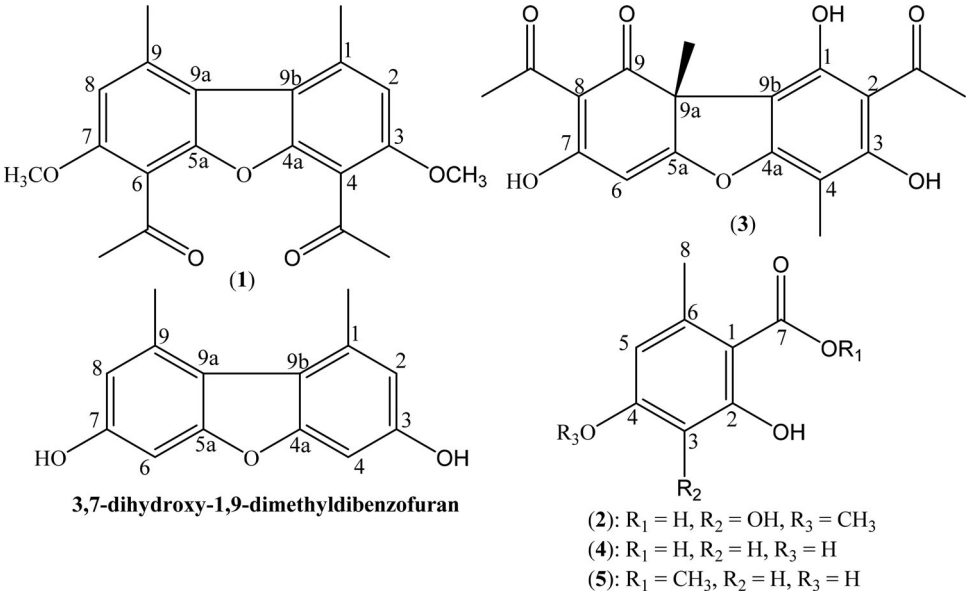


Figure 1. Chemical structures of compounds **1**–**5** and 3,7-dihydroxy-1,9-dimethyldibenzofuran.

at δ_C 96.6 (C-4, C-6) in 3,7-dihydroxy-1,9-dimethyldibenzofuran (Tanahashi et al. 2001). Additionally, four downfield-shifted carbons of C-4, C-6 [δ_C 132.8 in **1**, δ_C 96.6 in 3,7-dihydroxy-1,9-dimethyldibenzofuran (Tanahashi et al. 2001)], and C-3, C-7 [δ_C 161.7 in **1**, δ_C 156.9 in 3,7-dihydroxy-1,9-dimethyldibenzofuran (Tanahashi et al. 2001)] (Table S1), which illustrated two acetyl and two methoxy moieties were attached to be those carbons, C-4, C-6, C-3 and C-7, respectively. Further, these connections were clearly testified by correlations between protons at δ_H 3.95 (3-OC$\underline{H}_3$, 7-OC$\underline{H}_3$) and carbons at δ_C 161.7 (C-3, C-7), between protons at δ_H 2.43 (4-COC$\underline{H}_3$, 6-COC$\underline{H}_3$) and carbons at δ_C 199.3 (4-$\underline{C}$OCH$_3$, 6-$\underline{C}$OCH$_3$), 132.8 (C-4, C-6), between protons at δ_H 2.47 (1-C$\underline{H}_3$, 9-C$\underline{H}_3$) and carbons at δ_C 113.6 (C-9a, C-9b), 112.2 (C-2, C-8), between protons at δ_H 7.06 (2H, *s*, H-2, H-8) and carbons at δ_C 113.6 (C-9a, C-9b) in HMBC (Figure S1). Therefore, the structure of **1** was assigned as 4,6-diacetyl-3,7-dimethoxy-1,9-dimethyldibenzofuran, named usneaceratin A.

Compound **2** was obtained as a white solid. The molecular formula was identified as $C_9H_{10}O_5$ by HR-APCI-MS data ($[M-H]^-$ *m/z* 197.0452, calcd. for $C_9H_9O_5$, 197.0444). The ^{13}C & ^1H-NMR data of **2** (Table S1) detailed nine signals according to one carbonyl carbon at δ_C 162.6 (C-7), three oxygenated aromatic carbons at δ_C 148.3 (C-2), 137.6 (C-3), and 153.4 (C-4), two quaternary aromatic carbons at δ_C 114.9 (C-1) and 133.2 C-6), one methine aromatic carbon at δ_C 113.1 (C-5)/δ_H 6.82 (H-5), one oxygenated methyl carbon at δ_C 56.6 (4-OC$\underline{H}_3$)/δ_H 3.87 (4-OC$\underline{H}_3$) and one methyl carbon at δ_C 20.5 (C-8)/δ_H 2.39 (H-8). Those data of **2** confirmed a benzoic acid framework similar orsellinic acid (**4**), except for the appearance of two carbons at δ_C 137.6 (C-3), 56.6 (4-O$\underline{C}$H$_3$), and the multiplicity of proton at δ_H 6.82 (1H, *s*, H-5) in **2**, and the disappearing of one methine aromatic carbon at δ_C 100.3 (C-3) in **4** (Table S1), which were attested a pentasubstituted benzene system bearing one carboxyl, two hydroxyl, one methoxy and one methyl groups. The HMBC spectrum of **2** (Figure S1) supported correlations

between protons at δ_H 2.39 (H-8) and carbons at δ_C 114.9 (C-1), 113.1 (C-5), 162.6 (C-7), between proton at δ_H 6.82 (H-5) and carbons at δ_C 114.9 (C-1), 100.3 (C-3), 153.4 (C-4), between protons at δ_H 3.87 (4-O$\underline{CH_3}$) and carbon at δ_C 153.4 (C-4), in addition, proton H-5 (δ_H 6.82) was close in space to protons H-8 (δ_H 2.39) and 4-O$\underline{CH_3}$ (δ_H 3.87) in NOESY (Figure S1), were illuminated the arrangement of these substituents in the benzene nucleus. Thus, the structure of **2** was assigned as 3-hydroxy-4-*O*-methylorsellinic acid, named usneaceratin B.

All separated compounds were examined for the α-glucosidase inhibition. Compound (**2**) exhibited stronger α-glucosidase inhibition than acarbose (IC_{50} value of 41.8 and 214.50 μM, respectively).

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance III spectrometer, at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, using residual solvent signal as internal reference: δ_H 2.50 and δ_C 39.5 for DMSO-d_6; δ_H 2.05 and δ_C 30.6 for acetone-d_6. The HR-ESI-MS were recorded on a HR-ESI-MS MicroOTOF–Q mass spectrometer or on a LC-Agilent 1100 LC-MSD Trap spectrometer. TLC was carried out on precoated silica gel 60 F_{254} or silica gel GF_{254} (Merck, Kenilworth, NJ). Spots were visualised by spraying with 10% aqueous H_2SO_4 or 5% ferric chloride solutions followed by heating. Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm, Himedia).

3.2. Lichen material

The thalli of the lichen *U. ceratina* Arch were separated from the bark of various trees at 109 m altitude at Paksong town (15°11′00″ N, 106°14′00″ E), Paksong district, Champasack province, Laos in April 2015. The scientific name of the plant was authenticated by Dr. Harrie J. M. Sipman, Freie Universitaet, Berlin, Germany. A voucher specimen (US – B030) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University – Ho Chi Minh City, Vietnam.

3.3. Extraction and isolation

The fresh lichen thalli (1.60 kg) were cleaned under running tap water and air-dried. The ground powder (1.15 kg) was macerated with acetone at room temperature. After filtration, the solvent was evaporated under reduced pressure to dryness to afford the crude acetone residue (163.0 g). This crude extract was subjected to silica gel solid-phase extraction and eluted consecutively with chloroform, ethyl acetate, acetone and methanol to provide chloroform (C, 60.0 g), ethyl acetate (EA, 12.0 g), acetone (Ace, 55.0 g) and methanol (M, 3.5 g) fractions, respectively.

The ethyl acetate extract (12.0 g) was subjected to silica gel column chromatography, eluted by the solvent system of chloroform–methanol with increasing methanol to get seven fractions, coded EA1 – EA7. The fraction EA3 (1.5 g) was rechromatographed, eluted with chloroform–methanol (98:2) to deliver **1** (5.0 mg), and **3** (6.5 mg).

The fraction EA4 (3.8 g) was separated by silica gel chromatographic column using chloroform–methanol–acetic acid (98:2:1 drop) to give **2** (6.0 mg), **4** (5.0 mg) and **5** (5.0 mg).

3.3.1. Usneaceratin A *(1)*

White solid. HR-ESI-MS: negative *m/z* 339.1252 $[M-H]^-$ (calcd for $C_{20}H_{19}O_5$, 339.1227); ^{1}H-NMR (DMSO-d_6, 500 MHz, *J* in Hz) and ^{13}C-NMR (acetone-d_6, 125 MHz): see Table S1.

3.3.2. Usneaceratin B *(2)*

White solid. HR-APCI-MS: negative *m/z* 197.0452 $[M-H]^-$ (calcd for $C_9H_9O_5$, 197.0444). ^{1}H-NMR (acetone-d_6, 500 MHz, *J* in Hz) and ^{13}C-NMR (acetone-d_6, 125 MHz): see Table S1.

3.4. α-Glucosidase inhibition assay

All purified compounds (**1**–**5**) were evaluated inhibitory effect on α-glucosidase enzyme. Acarbose was used as the positive control. The details were similar to those presented in our previous paper (Nguyen et al. 2015, Nguyen, Le, Phan, Bui, Mai 2016; Nguyen, Le, Phan, Bui, Pham, et al. 2016). An aliquot of 25 μL of the substrate solution (*p*-nitrophenyl-α-D-glucopyranoside 3 mM), add 25 μL of the enzyme solution (α-glucosidase enzyme 0.2 U/mL in 0.01 M phosphate buffer solution, pH = 7) were added to 625 μL of the sample solution (compounds **1**–**5**). Those solutions were incubated at 37 °C for 30 min, and stopped by using 375 μL of Na_2CO_3 (0.1 M), further, measured the optical density at 401 nm. The IC_{50} value was calculated as the concentration of α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity.

Acknowledgements

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 authentication of the scientific name for the studied species.

Disclosure statement

No potential conflict of interest was by reported the authors.

References

Ahmadjian V, Hale ME. 1973. The lichens. New York and London: Academic Press. ISBN 9780120449507.

Bui VM, Duong TH, Chavasiri W, Nguyen KPP, Huynh BLC. 2020. A new depsidone from the lichen *Usnea ceratina* Arch. Nat Prod Res.doi:10.1080/14786419.2020.1828405.

Huneck S, Yoshimura I. 1996. Identification of lichen substances. New York (NY): Springer-Verlag Berlin Heidelberg. ISBN 978-3-642-85245-9.

Huynh BLC, Bui VM, Nguyen KPP, Pham NKT, Nguyen TP. 2020. Three new diphenyl ethers from the lichen *Parmotrema praesorediosum* (Nyl.) Hale (Parmeliaceae). Nat Prod Res.

Huynh BLC, Le HD, Takenaka Y, Tanahashi T, Nguyen KPP. 2016. New phenolic compounds from the lichen *Parmotrema praesorediosum* (Nyl.) Hale (Parmeliaceae). Magn Reson Chem. 54(1): 81–87.

Huynh BLC, Pham NKT, Nguyen TP. 2021. Vinapraesorediosic acids D and E from the lichen *Parmotrema praesorediosum* (Nyl.) hale. Phytochem Lett. 41:61–64.

Nash TH. III, 2008. Lichen Biology. New York (NY): Cambridge University Press. ISBN 9780511790478.

Nguyen TP, Le TD, Phan NM, Bui TD, Mai DT. 2016. Triterpene saponins with α-glucosidase inhibition and cytotoxic activity from the leaves of *Schefflera sessiliflora*. J Asian Nat Prod Res. 18(6):542–549.

Nguyen TP, Le TD, Phan NM, Bui TD, Pham NKT, Do TML, Nguyen DT, Mai DT. 2016b. A new dihydrofurocoumarin from the fruits of *Pandanus tectorius* Parkinson ex Du Roi. Nat Prod Res. 30(21):2389–2395.

Nguyen TP, Tran TTV, Mai DT, Le TD, Phan NM, Bui TD. 2015. New C_{20}-gibberellin diterpene from the leaves of *Schefflera sessiliflora* De P. V. Nat Prod Res. 29(15):1432–1436.

Prateeksha P, Paliya BS, Bajpai R, Jadaun V, Kumar J, Kumar S, Upreti DK, Singh BR, Nayaka S, Joshi Y, et al. 2016. The genus *Usnea*: a potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology. RSC Adv. 6(26):21672–21696.

Tanahashi T, Takenaka Y, Nagakura N, Hamada N. 2001. Dibenzofurans from the cultured lichen mycobionts of *Lecanora cinereocarnea*. Phytochem. 58(7):1129–1134.