NATURAL PRODUCT RESEARCH
2022, VOL. 36, NO. 8, 1934–1940
https://doi.org/10.1080/14786419.2020.1837818

Three new diphenyl ethers from the lichen *Parmotrema praesorediosum* (Nyl.) Hale (Parmeliaceae)

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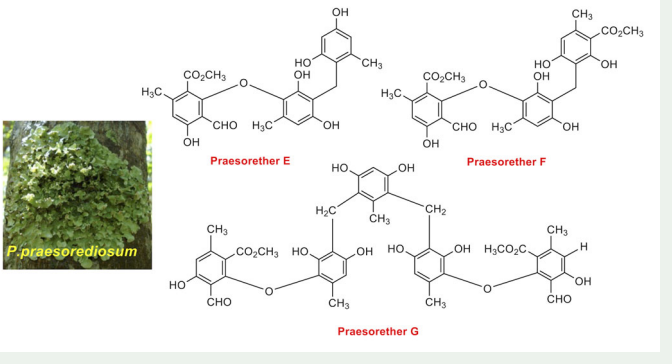
ABSTRACT

Three new diphenyl ethers, named praesorethers E, F and G (**1**, **2** and **3**), were isolated from the lichen *Parmotrema praesorediosum*. Their chemical structures were elucidated on the basis of extensively spectroscopic analysis including HR-ESI-MS and NMR as well as comparison with previously published data. These compounds were evaluated for the cytotoxicity against three human cancer cell lines (HeLa, NCI-H460 and MCF-7) using SRB assay. As results, **1** and **2** exhibited weak cytotoxic activity against three tested cancer cell lines with the inhibitive percentage of 64–79.9% at the concentration of 100 μg/mL while **3** was inactive.



ARTICLE HISTORY
Received 29 July 2020
Accepted 9 October 2020

KEYWORDS
Parmotrema praesorediosum; Parmeliaceae; praesorether; diphenyl ether; cytotoxicity

1. Introduction

Lichens are symbiotic products of a mycobiont (fungal partner) and photobiont (algal partner) and are known to produce a range of secondary metabolites, of which some

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1837818.

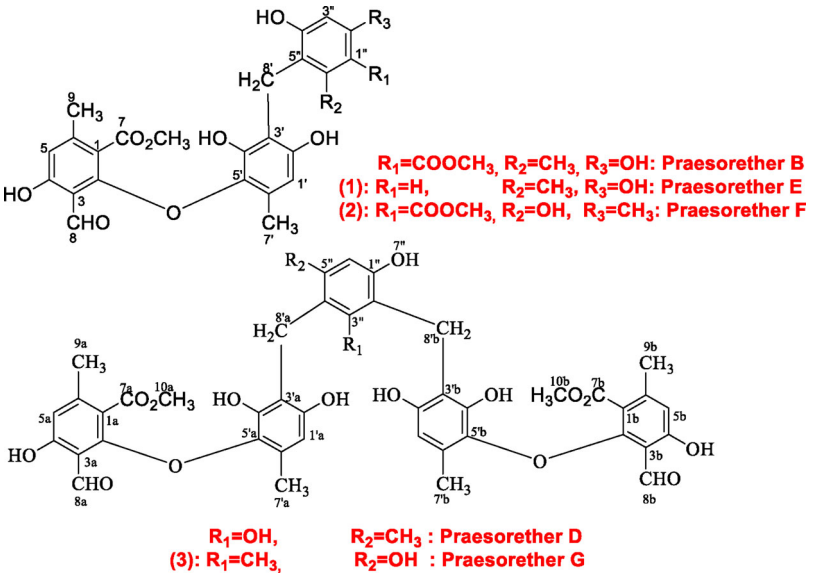

Figure 1. Chemical structures of compounds **1**–**3** and praesorethers B, D.

are unique to lichen symbiosis, for example, depsides, depsidones and diphenyl ethers. These secondary metabolites have shown an impressive range of biological activities, including antibiotic, antifungal, antiviral, antitumor and anticancer (Ahmadjian and Hale 1973; Huneck and Yoshimura 1996; Nash 2008). However, lichens remain a relatively unexplored source of biologically active compounds when compared with higher plants.

Cancer chemoprevention is a strategy for reducing cancer mortality and involves the prevention, delay or reversal of cancer by the ingestion of dietary or pharmaceutical agents capable of modulating the process of carcinogenesis (Wattenberg 1985; Hong and Sporn 1997).

In an attempt to obtain structurally unusual compounds with potential cancer chemoprevention activity from plants sources (Nguyen et al. 2016; Mai et al. 2019; Ngo et al. 2017, 2019, 2020) as well as from the lichen (Huynh et al. 2010, 2016), this paper concentrated on the isolation, structural elucidation and cytotoxicity against HeLa, NCI-H460 and MCF-7 cancer cell lines of three new diphenyl ethers (**1**–**3**) from the lichen *Parmotrema praesorediosum* (Nyl) Hale collected in Tan Phu forest, Dong Nai province, Vietnam.

2. Results and discussion

The chloroform and ethyl acetate extracts of *P. praesorediosum* collected in Tan Phu forest, Dong Nai province, were applied to column chromatography over silica gel normal-phase to afford three new diphenyl ethers, named praesorethers E, F and G (**1**–**3)** (Figure 1).

Compound **1** was isolated as a yellow solid. Its molecular formula was established as $C_{25}H_{24}O_9$ by HR-ESI-MS data ($[M-H]^-$ *m/z* 467.1329, calcd. for $C_{25}H_{23}O_9$, 467.1342). The IR absorptions showed the presence of the hydroxyl (3351 cm^{-1}), carbonyl

(1703 cm^{-1}) and olefin (1619 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** (supporting information Table S1) displayed one chelated hydroxyl proton at δ_H 12.10 (1H, *s*, 4-OH), one aldehyde proton at δ_H 10.44 (1H, *s*, H-8), four aromatic protons at δ_H 6.50 (1H, *s*, H-5), 6.27 (1H, *d*, $J = 2.5$ Hz, H-3″), 6.25 (1H, *d*, $J = 2.5$ Hz, H-1″) and 6.22 (1H, *s*, H-1′), one methoxy signal at δ_H 3.13 (3H, *s*, 7-OCH_3), two non-equivalent methylene protons at δ_H 3.88 (1H, *brs*, H-8′a), 3.86 (1H, *brs*, H-8′b) and three methyl groups at δ_H 2.39 (3H, *s*, H-7″), 2.13 (3H, *s*, H-9) and 2.01 (3H, *s*, H-7′). The combination of ^{13}C NMR and HSQC spectra of **1** (supporting information Table S1) revealed 25 carbons including one formyl group at δ_C 195.6 (C-8), one carbonyl carbon at δ_C 166.7 (C-7), 18 aromatic carbons in the range of 101–165 ppm with seven of which were oxygenated and four of them were aromatic methine carbons at δ_C 112.8 (C-5), 110.9 (C-1″), 108.8 (C-1′) and 101.0 (C-3″), one oxymethyl carbon at δ_C 51.9 (7-OCH_3), one methylene carbon at δ_C 20.3 (C-8′) and three methyl groups at δ_C 20.8 (C-7″), 20.5 (C-9) and 16.6 (C-7′). These data indicated that **1** had the same skeleton with praesorether B possessed three aromatic rings in which the rings A and B are connected through an ether linkage and the rings B and C are jointed together *via* a methylene bridge at its C-3′ and C-5″ (Huynh et al. 2016). A comparison of the ^{1}H and ^{13}C NMR data of **1** with praesorether B (supporting information Table S1) revealed that the methoxycarbonyl group in the latter was replaced by an aromatic methine proton H-1″ (δ_H 6.25, *d*, $J = 2.5$ Hz) which *meta*-coupled with proton H-3″ (δ_H 6.27, *d*, $J = 2.5$ Hz) in the C-ring. This was supported by HMBC correlations (supporting information Figure S1) of this aromatic proton H-1″ with carbons at δ_C 157.1 (C-2″), 116.7 (C-5″) and 20.8 (C-7″). According to the above-mentioned analysis, the structure of **1** was established as 1″-demethoxycarbonylpraesorether B or praesorether E.

Compound **2** was obtained as a yellow solid. The molecular formula was established as $C_{27}H_{26}O_{11}$ by HR-ESI-MS data ($[M-H]^-$ *m/z* 525.1380, calcd. for $C_{27}H_{25}O_{11}$, 525.1397). The comparison of ^{1}H and ^{13}C-NMR data of **2** (supporting information Table S1) and praesorether B (Huynh et al. 2016) showed that they possessed similar A and B rings and some differences in the position of substituents in their C rings with one downfield-shifted carbon due to C-3″ (δ_C 110.0), and two upfield-shifted carbons of C-1″ (δ_C 105.0), and C-5″ (δ_C 113.4). These revealed that they were positional isomers. In praesorether B, the carbon C-3″ (δ_C 101.5) was deshielded by two adjacent oxygenated carbons C-2″ (δ_C 161.5), and C-4″ (δ_C 160.0), and the carbon C-5″ (δ_C 119.5) was in the middle position of one oxygenated carbon C-4″ (δ_C 160.0), and a carbon joining to a methyl group, C-6″ (δ_C 142.5), and this arrangement was a little modified in **2** in which the carbon C-5″ (δ_C 113.4) was deshielded by two adjacent oxygenated carbons C-4″ (δ_C 161.0), and C-6″ (δ_C 1412.0), and the carbon C-3″ (δ_C 111.0) was in the middle position of one oxygenated carbon C-4″ (δ_C 160.0) and a carbon joined to a methyl group, C-2″ (δ_C 160.0). This structure was in good agreement with HMBC spectrum (supporting information Figure S1) with correlations of two methylene protons, H_2-8′ at δ_H 3.88, to two oxygenated aromatic carbons at δ_C 161.0 (C-4″), 142.0 (C-6″) and 113.4 (C-5″) and of the aromatic methine proton H-3″ at δ_H 6.38 to one oxygenated aromatic carbon at δ_C 161.0 (C-4″), and to the carbon linked to a methyl group (C-2″) at δ_C 160.0 and also to this methyl group at δ_C 24.1 (C-8″). These data assigned that one methyl and two hydroxyl groups were at C-2″, C-4″ and C-6″ of ring C in **2**,

respectively. Based on these supporting evidences, the structure of **2** was suggested as shown, named praesorether F.

Compound **3** was obtained as a yellow solid. The molecular formula was established as $C_{43}H_{40}O_{16}$ by HR-ESI-MS data ($[M+Na]^+$ *m/z* 835.2239, calcd. for $C_{43}H_{40}O_{16}Na$, 835.2214). The comparison of ^1H and ^{13}C NMR data of **2** and **3** (supporting information Table S1) showed the similarities. However, there were differences in their HR-MS data. If the molecular formula of **2** was $C_{27}H_{26}O_{11}$, the one of **3** was $C_{43}H_{40}O_{16}$. Interestingly, the HR-MS value of **3** was identical with that of praesorether D (Huynh et al. 2016). These data deduced that **3** and praesorether D were positional isomers with the difference in the position of substituents in the C-ring. The methyl and two hydroxyl groups were in a symmetrical arrangement in the C-ring of **3** while in praesorether D, it was an asymmetrical one.

Indeed, in the C-ring of **3**, the carbon C-6″ (δ_C 100.8) was strongly deshielded by two adjacent oxygenated carbons, C-1″ and C-5″ (the two were at δ_C 153.5) while in praesorether D, the carbon C-6″ (δ_C 109.0) was adjacent to one oxygenated carbon C-1″ (δ_C 152.9) and to a carbon linked to a methyl group, C-5″ (δ_C 137.8). These data were well supported by HMBC correlations (supporting information Figure S1) of the sole aromatic proton at δ_H 6.42 (H-6″) to two oxygenated aromatic carbons at δ_C 153.5 (C-1″, C-5″), of the symmetrical methylene protons H_2-8′(of two moieties a and b) to carbons C-3″ (δ_C 141.4), and C-1″, C-5″ (δ_C 153.5). Based on these supporting evidences, the structure of **3** was proved as shown, named praesorether G.

These compounds were evaluated for the cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer) and MCF-7 (human breast cancer) cell lines using SRB assay. As results, compounds **1** and **2** expressed weak cytotoxic effect against all tested cancer cell lines (at the concentration of 100 μg/mL, percent of cytotoxicity ranged from 64.3% to 79.9%), while compound **3** was inactive.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance III spectrometer, at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR, using residual solvent signal as internal reference: chloroform-*d*, δ_H 7.26, δ_C 77.16; acetone-d_6, δ_H 2.05, δ_C 206.31 and 30.6. 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 $60F_{254}$ or silica gel GF_{254} (Merck). 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. Plant material

The lichen thalli of *P. praesorediosum* were collected on the bark of *Dipterocarpus* sp. at Tan Phu forest, Dong Nai province, Vietnam in June 2009. The geographical location where the lichen was collected is at an altitude of 110 m, 11°20′–11°50′ N and 107°09′—107°35′ E. The botanical species of *P. praesorediosum* (Nyl.) Hale (synonym of

P. praesorediosa Nyl.) was identified by Dr. Vo Thi Phi Giao, Faculty of Biology, University of Science, National University – Ho Chi Minh City. A voucher specimen (No US-B020) was deposited in the Herbarium of The Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University – Ho Chi Minh City, Vietnam.

3.3. Extraction and isolation

The thallus material (5.0 kg) was washed under flow of tap water and then was air-dried at ambient temp. to obviate thermally induced decomposition prior to be ground into a fine powder. The ground powder sample (3.0 kg) was macerated by methanol at room temperature to afford a crude methanol extract (450 g). This crude one (450 g) was applied to silica gel solid phase extraction, successively eluted with the following solvents: petroleum ether (60–90 °C) (PE), chloroform (C), ethyl acetate (EA), acetone (A) and methanol (M) to afford corresponding extracts: extract PE (40 g), extract C (105 g), extract EA (50 g), extract A (45 g) and extract M (37 g).

The chloroform extract (105 g) was subjected to silica gel column chromatography, eluted by the solvent system of petroleum ether–ethyl acetate with increasing ethyl acetate to give 23 fractions, coded C1–C23. The fraction C13 (5.7 g) was rechromatographed, eluted with petroleum ether–chloroform (8:2) to give **2** (5.0 mg).

The ethyl acetate extract (50 g) was applied to silica gel column chromatography, eluted with the solvent systems of chloroform–methanol with increasing methanol to give seven fractions, coded EA1–EA7. The fraction EA4 (14.4 g) was rechromatographed, eluted with chloroform–methanol (95:5) to give **1** (10.0 mg) and **3** (9.0 mg).

***Praesorether E* (1):** yellow solid. IR (KBr) υ_{max}: 3,37,93,35,11,70,31,64,00,000 cm^{-1}; HR-ESI-MS: negative *m/z* 467.1329 $[M-H]^-$ (calcd for $C_{25}H_{23}O_9$, 467.1342); ^1H NMR (acetone-d_6, 500 MHz, *J* in Hz): 12.10 (1H, *s*, 4-OH), 10.44 (1H, *s*, H-8), 6.50 (1H, *s*, H-5), 6.27 (1H, *d*, 2.5, H-3″), 6.25 (1H, *d*, 2.5, H-1″), 6.22 (1H, *s*, H-1′), 3.88 (1H, *brd*, H-8′a), 3.86 (1H, *brd*, H-8′b), 3.12 (3H, *s*, 7-OCH_3), 2.39 (3H, *s*, H-7″), 2.13 (3H, *s*, H-9), 2.01 (3H, *s*, H-7′). ^{13}C NMR (acetone-d_6, 125 MHz): 195.7 (C-8), 166.7 (C-7), 164.2 (C-4), 159.1 (C-2), 157.1 (C-2″), 155.7 (C-4″), 153.4 (C-2′), 149.2 (C-4′), 148.2 (C-6), 140.9 (C-6″), 135.7 (C-5′), 129.9 (C-6′), 116.6 (C-5″), 115.6 (C-1), 112.8 (C-5), 113.4 (C-3′), 111.0 (C-3), 110.9 (C-1″), 108.8 (C-1′), 101.0 (C-3″), 51.9 (7-O**C**H_3), 20.8 (C-7″), 20.5 (C-9), 20.3 (C-8′), 16.6 (C-7′).

Praesorether F (**2**): yellow solid. HR-ESI-MS: negative *m/z* 525.1380 $[M-H]^-$ (calcd for $C_{27}H_{25}O_{11}$, 525.1397). ^1H NMR ($CDCl_3$, 500 MHz, *J* in Hz): 13.44 (1H, *s*, 2″-OH), 12.05 (1H, *s*, 4-OH), 10.32 (1H, *s*, H-8), 6.54 (1H, *s*, H-5), 6.38 (1H, *s*, H-3″), 6.31 (1H, *s*, H-1′), 3.94 (3H, *s*, 7″-OCH_3), 3.88 (2H, *s*, H-8′), 3.28 (3H, *s*, 7-OCH_3), 2.45 (3H, *s*, H-8″), 2.21 (3H, *s*, H-9), 1.99 (3H, *s*, H-7′). ^{13}C NMR ($CDCl_3$, 125 MHz): 193.8 (C-8), 173.1 (C-7″), 167.5 (C-7), 164.1 (C-4), 161.0 (C-4″), 160.0 (C-2″), 158.3 (C-2), 152.3 (C-2′), 147.6 (C-6), 145.5 (C-4′), 142.0 (C-6″), 136.3 (C-5′), 129.5 (C-6′), 115.3 (C-1), 114.0 (C-5), 113.4 (C-5″), 112.2 (C-3′), 111.1 (C-1′), 111.1 (C-3″), 110.9 (C-3), 105.0 (C-1″), 52.4 (C-7″), 24.1 (C-8″), 52.3 (7-O**C**H_3), 20.9 (C-9), 17.3 (C-8′), 16.8 (8″-OCH_3).

Praesorether G (**3**): yellow solid. IR (KBr) υ_{max}: 3,45,43,41,93,38,21,72, 00,00,00,000 cm^{-1}. HR-ESI-MS: positive *m/z* 835.2239 $[M+Na]^+$ (calcd for $C_{43}H_{40}O_{16}Na$,

835.2214). ^{1}H NMR (acetone-d_6, 500 MHz, *J* in Hz): 12.08 (1H, *s*, 4-OH), 10.40 (1H, *s*, H-8), 6.50 (1H, *s*, H-5), 6.42 (H, *s*, H-3″), 6.19 (H, *s*, H-1′), 3.94 (1H, *brd*, H-8′a), 3.92 (1H, *brd*, H-8′b), 3.10 (3H, *s*, 7-OCH_3), 2.53 (3H, *s*, H-7″), 2.13 (3H, *s*, H-9), 1.99 (3H, *s*, H-7′). ^{13}C NMR (acetone-d_6, 125 MHz): 195.6 (C-8a, C-8b), 166.7 (C-7a, C-7b), 164.2 (C-4a, C-4b), 159.1 (C-2a, C-2b), 153.5 (C-2″, C-4″), 153.3 (C-2′a, C-2′b), 149.1 (C-4′a, C-4′b), 148.2 (C-6a, C-6b), 141.4 (C-6″), 135.6 (C-5′a, C-6′a, C-5′b, C-6′b), 118.6 (C-1″, C-5″), 115.7 (C-1a, C-1b), 113.2 (C-3′a, C-3′b), 112.7 (C-5a, C-5b), 110.9 (C-3a, C-3b), 108.7 (C-1′a, C-1′b), 100.8 (C-3″), 51.9 (7a-O**C**H_3, 7b-O**C**H_3), 20.7 (C-8′a, C-8′b), 20.5 (C-9a, C-9b), 16.8 (C-7″), 16.6 (C-7′a, C-7′b).

3.4. Biological assay

Compounds **1–3** were subjected to a cytotoxic evaluation against HeLa (human epithelial carcinoma), MCF-7 (human breast cancer) and NCI-H460 (human lung cancer) cell lines. The tested samples were performed at a concentration of 100 μg/mL using sulforhodamine B (SRB) assay with camptothecin as the positive control. The details were similar to those presented in our previous paper (Skehan et al. 1990).

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 authentification of the scientific name for the studied species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

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

Hong WK, Sporn MB. 1997. Recent advances in chemoprevention of cancer. Science. 278(5340): 1073–1077.

Huneck S, Yoshimura I. 1996. Identification of lichen substances. New York: Springer-Verlag Berlin Heidelberg.

Huynh BLC, Duong TH, Tanahashi T, Nguyen KPP. 2010. Contribution to the study on chemical constituents of the lichen *Parmotrema praesorediosum* (Nyl.) Hale, Parmeliaceae. Vietnam J Chem. 48:332–337.

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.

Mai DT, Ngo TN, Nguyen NTL, Ngo QL, Phan NM, Bui TD, Dang VS, Tran CL, Pham NKT, Tran NMA, et al. 2019. Cytotoxic activity and phytochemical composition of *Stereospermum binhchauensis* V.S. Dang leaves. Nat Prod Res. 1–3.

Nash TH, III. 2008. Lichen biology. New York: Cambridge University Press.

Ngo TN, Nguyen NDP, Nguyen NTL, Pham NKT, Phan NM, Bui TD, Dang VS, Tran CL, Mai DT, Nguyen TP. 2020. Markhasphingolipid A, new phytosphingolipid from the leaves of *Markhamia stipulata* var. *canaense* V.S. Dang. Nat Prod Res. 34(13):1820–1826.

Ngo TN, Phan NM, Bui TD, Dang VS, Tran CL, Mai DT, Nguyen TP. 2017. Cytotoxic cycloartane triterpenoids from the leaves of *Markhamia stipulata* var. *canaense*. Phytochem Lett. 22: 251–254.

Ngo TN, Vo CT, Pham NKT, Phan NM, Bui TD, Ngo QL, Dang VS, Tran CL, Mai DT, Nguyen TP. 2019. Markhacanasin C, cycloartane triterpenoid from the leaves of *Markhamia stipulata* var. *canaense* V.S. Dang. Nat Prod Res. 33(2):174–179.

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.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 82(13):1107–1112.

Wattenberg LW. 1985. Chemoprevention of cancer. Cancer Res. 45(1):1–8.