New terpenoids from the corticioid fungus *Punctularia atropurpurascens* and their antimycobacterial evaluation

By: Daniel Acero, Firoz Shah Tuglak Khan, Abraham J. Medina-Ortiz, Isabel Rivero-Cruz, Huzefa A. Raja, Laura Flores-Bocanegra, Carlos A. Fajardo-Hernández, Baojie Wan, Scott G. Franzblau, Shabnam Hematian, Mario Figueroa

Daniel Acero, Firoz Shah Tuglak Khan, Abraham J. Medina-Ortiz, Isabel Rivero-Cruz, Huzefa A. Raja, Laura Flores-Bocanegra, Carlos A. Fajardo-Hernández, Baojie Wan, Scott G. Franzblau, Shabnam Hematian, Mario Figueroa. New Terpenoids from the Corticioid Fungus *Punctularia atropurpurascens* and their Antimycobacterial Evaluation. *Planta Medica*. 2022. https://doi.org/10.1055/a-1786-8072

Abstract:

Chemical investigation of *Punctularia atropurpurascens* strain HM1 (Punctulariaceae), a corticioid isolated from a decorticated piece of *Quercus* bark collected in Bosque de Tlalpan, Mexico City, led to the isolation of a new drimane, 1-*α*-hydroxy-isodrimenine (**1**) and a new tetrahydroxy kauranol, 16-hydroxy-phlebia-*nor*-kauranol (**2**), together with the known *N*-phenylacetamide (**3**). Structures of all compounds were elucidated by spectroscopic and spectrometric methods, and the absolute configuration of **1** and **2** was confirmed via single-crystal X-ray crystallography. The isolated compounds showed modest antimycobacterial activity.

Keywords: *Punctularia atropurpurascens* | corticioid | drimane | *nor*-kaurane | antitubercular activity | Punctulariaceae

Article:[1]

Introduction

Punctularia atropurpurascens (Berk. & Broome) Petch is a member of the Punctulariaceae that produces a characteristic purplish exudate [1, 2]. This saprotrophic basidiomycete with a fluffy, membranous, and waxy hymenial surface grows on the surface of decaying wood, mainly oak species (*Quercus*) [1, 2]. *Punctularia atropurpurascens* and *P. strigosozonata*, the only two species of this genus that occur in North America [3], are known to produce kauran-type diterpenes (e.g., phlebiakauranol aldehyde and the corresponding alcohol), and p-terphenyls derivatives (e.g., phlebiarubrone), respectively [4, 5]. To the best of our knowledge, no further chemical studies have been conducted on fungus of this genus.

[1] Dedicated to Professor Dr. A. Douglas Kinghorn on the occasion of his 75th birthday.

As part of ongoing work to uncover new bioactive compounds from nature [6], *P. atropurpurascens* strain HM1 was isolated from a decorticated piece of *Quercus* bark in Bosque de Tlalpan, Mexico City, Mexico. From the axenic cultures on potato dextrose agar (PDA) plates, fermentation cultures in rice medium were prepared and subjected to chemical analysis. Herein, we describe the isolation and structure elucidation of two new terpenoids along with a known compound, from the organic extract. In addition, the antimycobacterial activity of each isolated compound was assessed. Finally, molecular identification of the strain was performed by phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer region. This work represents the first chemical and biological study of a *P. atropurpurascens* strain isolated from Mexico.

Results and Discussion

The strain HM1 was isolated from a piece of *Quercus* bark collected in Bosque de Tlalpan in Mexico City (Fig. 1a). Interestingly, when the strain was grown on PDA medium, it did not produce the purple exudate as it does in its natural habitat; however, colonies were white/purple when young and became dark brown after 7 days of growth (Fig. 1b, c). Using morphological characteristics and molecular studies (based on ITS1-5.8S-ITS2 sequence data), this strain was identified as the basidiomycete *P. atropurpurascens* (Punctulariaceae) (Supporting Information).

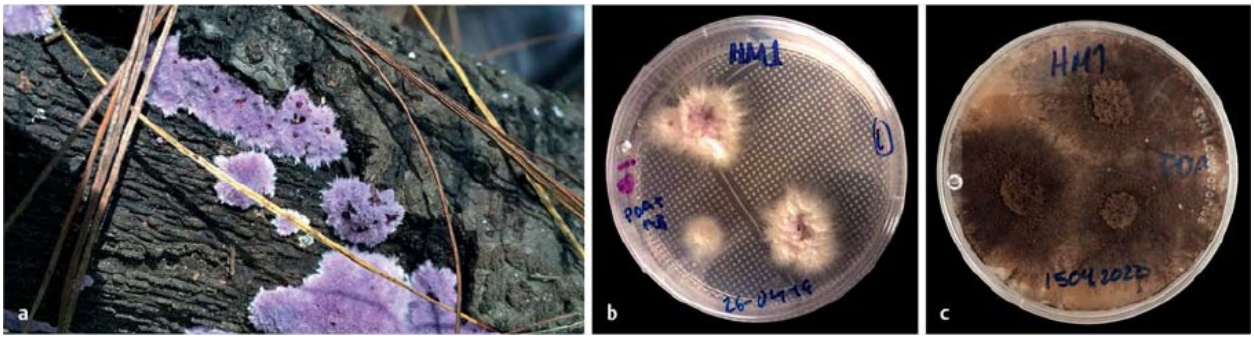

Fig. 1. a Strain HM1 growing on decorticated *Quercus* bark in Bosque de Tlalpan, Mexico City (2310 masl; 19°17′ 42.908″ N, 99°11′ 54.199″ W). **b** Three-day-old and **c** fourteen-day-old colonies of *P. atropurpurascens* strain HM1 grown on PDA.

Fractionation of the organic extract of *P. atropurpurascens* strain H1 using flash chromatography, followed by preparative RP-HPLC, afforded two undescribed natural products (1 and 2), together with N-phenylacetamide (3) (Fig. 2).

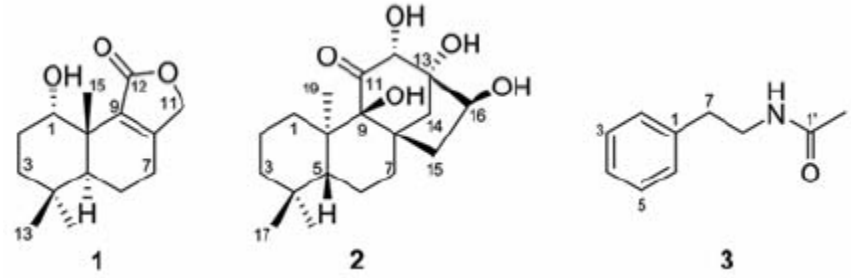

Fig. 2. Chemical structures of compounds 1-3 isolated from *P. atropurpurascens*.

Compound 1 (1.1mg) was isolated as colorless crystals. The molecular formula $C_{15}H_{22}O_3$ was deduced by HRESIMS ion peak at m/z 251.1640 $[M + H]^+$ (calculated for $C_{15}H_{23}O_3$, 251.1642, − 0.7 ppm), indicating an index of hydrogen deficiency of 5 (Fig. 1S, Supporting Information). Detailed analysis of the 1D and 2D NMR data (Table 1, Figs. 2S-7S, Supporting Information) revealed that the structure of 1 was a C-1 hydroxylated derivative of isodrimenine [5,7]. The ^{1}H NMR signals of 1 showed the presence of one hydroxy methine (δ_H 4.62, m), one methine (δ_H 1.73, m), one oxymethylene (δ_H 4.59, d, J = 6.6 Hz), four methylenes (δ_H 1.23-2.36), and three methyl groups (δ_H 0.89, 0.96, and 1.12), confirming the sesquiterpene skeleton (Fig. 2S, Supporting Information). ^{13}C NMR and HSQC spectra displayed fifteen carbons attributable to one carbonyl, three methyls, five methylenes (including one oxygenated), two methines (including one oxygenated), and four non-protonated carbons (Figs. 3S and 4S, Supporting Information). The methine at δ_C 69.3 in the ^{13}C NMR spectrum and the correlation observed from H-15 to C-1 in the HMBC spectrum supported the presence of the OH group at C-1 (Fig. 3 and Fig. 5S, Supporting Information). Furthermore, HMBC data showed the correlations from H-7 and H-11 to C-8 and C-9, confirming that the lactone ring is connected to the bicyclic part of the molecule at C-8 and C-9. Analysis of the ^{1}H-^{1}H COSY spectrum established the presence of two 6-membered ring spin systems: one from H-1 to H-3 and the other one from H-5 to H-7 (Fig. 3 and Fig. 6S, Supporting Information). Finally, the relative configuration of 1 was determined by a NOESY experiment (Figs. 7S, Supporting Information) and confirmed as 1S,5S,10S-1 by single-crystal X-ray diffraction analysis (Fig. 4 and Table 2S, Supporting Information). Thus, compound 1 was identified as a new drimane and given the trivial name 1-α-hydroxy-isodrimenine.

Table 1. NMR data of 1 (400 MHz for ^{1}H and 150 MHz for ^{13}C, $CDCl_3$, δ in ppm, J in Hz).

Position	δ_H, mult. (J)	δ_C, type	HMBC (H→C)
1	4.62, m	69.3, CH	3, 15
2	1.62, m 1.94, m	25.2, CH_2	4
3	1.23, m 1.71, m	34.9, CH_2	5, 13, 14
4	—	33.0, C	—
5	1.73, m	44.6, CH	1, 7, 10
6	1.64, m 1.94, m	18.0, CH_2	10
7	2.36, m	25.0, CH_2	5, 8, 9
8	—	161.9, C	—
9	—	134.0, C	—
10	—	40.5, C	—
11	4.59, d (6.6)	71.5, CH_2	8, 9, 12
12	—	173.5, C	—
13	0.89, s	21.2, CH_3	3, 5, 14
14	0.96, s	33.2, CH_3	3, 5, 13
15	1.12, s	21.4, CH_3	1, 5, 9

Table 2. NMR data of 2 (500 MHz for ^{1}H and 150 MHz for ^{13}C, methanol-d_4, δ in ppm, *J* in Hz).

Position	δ_H, mult. (*J*)	δ_C, type	HMBC (H→C)
1	1.08, m 1.69, m	32.2, CH_2	3, 5
2	1.43, m 1.50, m	19.5, CH_2	4, 10
3	1.15, m 1.34, m	42.1, CH_2	5, 17, 18
4		33.0, C	—
5	1.56, m	46.1, CH	7, 19
6	1.61, m	17.8, CH_2	5, 7
7	1.43, m 1.64, m	36.5, CH_2	5, 14
8	—	44.6, C	—
9	—	85.0, C	—
10	—	43.9, C	—
11	—	214.5, C	—
12	4.10, d (2.0)	74.5, CH	13, 16
13	—	80.3, C	—
14	1.66, m 1.92, m	41.4, CH_2	12, 16
15	1.36, m 2.90, m	40.4, CH_2	8, 16
16	4.07, dd (11.3, 5.1)	75.3, CH	12, 15
17	0.82, s	20.8, CH_3	3, 5, 18
18	0.85, s	32.8, CH_3	3, 5, 17
19	1.41, s	17.7, CH_3	1, 5, 9

Compound 2 (4.3mg) was isolated as white crystals. Its molecular formula, $C_{19}H_{30}O_5$, was established based on the HRESIMS ion peak at m/z 339.2165 $[M + H]^+$ (calculated for $C_{19}H_{31}O_5$, 339.2166, − 0.3 ppm), suggesting the presence of 5 degrees of unsaturation (Fig. 8S, Supporting Information). Additionally, the loss of four OH groups was deducted by the fragmentation pattern observed from the molecular ion in the MS ionization source. The ^{1}H NMR (Table 2, Fig. 9S, Supporting Information) spectrum revealed the presence of 13 different proton resonances, including two hydroxy methines at δH 4.07 (dd, *J* = 5.1, 11.3Hz) and 4.10 (d, *J* = 2.0 Hz), one aliphatic methine at δ_H 1.56 (m), three methyl singlets at δ_H 0.82, 0.85, and 1.41; and seven methylenes in the range of δ_H 1.10-2.90. The ^{13}C NMR and HSQC spectra (Table 2, Figs. 10S and 11S, Supporting Information) showed 19 carbon signals, corresponding to one carbonyl, seven methylenes, three methines (including two oxygenated), three methyls, two oxygenated sp^3 non-protonated carbons, and three quaternary carbons. These analyses suggested a nor-kaurane-type structure based on the reports from Anke et al. [4] and Lisy et al [8]. Comparison of the 2D NMR spectra (Figs. 11S-14S, Supporting Information) of 2 with those of phlebia-*nor*-kauranol isolated from the fungus *Phlebia strigozonata* [4,8], indicated that 2 has a hydroxyl group at C-16 (δ_C 74.5), instead of a carbonyl group. This conclusion was further supported by the HMBC correlations of H-14 to C-7, H-7 to C-5; H-16 to C-15; H-19 to C-9; and H-12 to C-13 (Fig. 3 and Fig. 13S, Supporting Information). Finally, the relative configuration of 2 was determined by a NOESY experiment (Figs. 14S, Supporting Information) and was successfully confirmed

through single-crystal X-ray crystallography as 5R,8R,9S,10R,12R,13S,16S-2 (Fig. 4). Therefore, this tetrahydroxy derivative 2 was given the trivial name of 16-hydroxy-phlebia-*nor*-kauranol and represents a new compound.

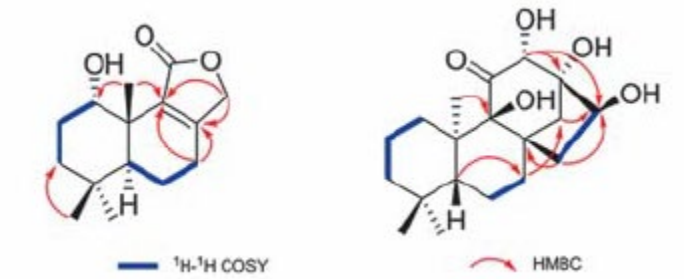

Fig. 3. Key HMBC (H→C) and ^{1}H-^{1}H COSY correlations of compounds 1 and 2.

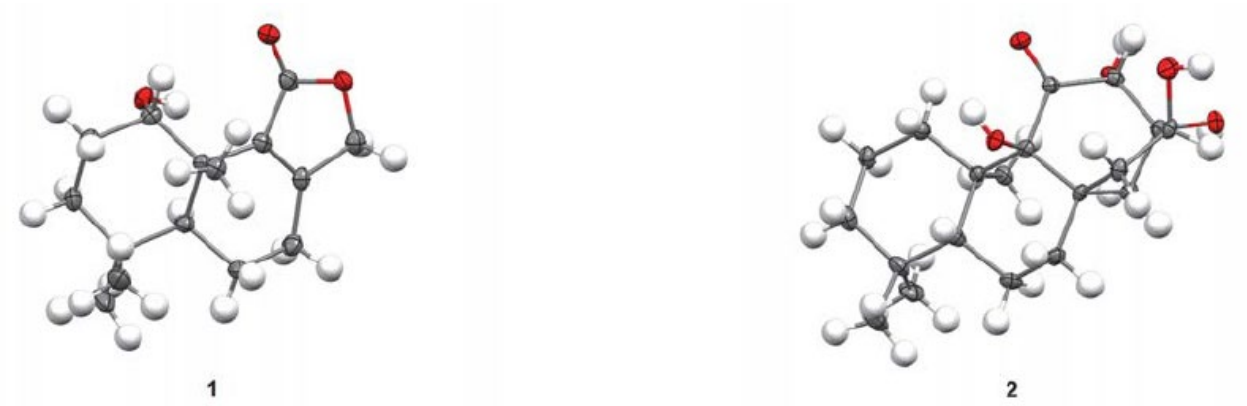

Fig. 4. Displacement ellipsoid plots (50% probability level) of 1 and 2 at 100(2) K.

Compound 3 (2.9mg) was identified as *N*-phenylacetamide by comparing its MS and NMR data (Table 1S and Figs. 15S-17S, Supporting Information) with those reported in the literature [9-11]. This compound has been isolated from the edible basidiomycete *Grifola frondosa* [9], the marine endophyte fungus Y26-02 isolated from *Clerodentrum inerme* [10], and the ascomycete *Tricladium* sp. GU288817 [11]. It is important to mention that to date, this compound has not been described as a constituent of any species of the genus *Punctularia*.

Finally, the *in vitro* antimycobacterial activity of 1-3 was assessed against *M. tuberculosis* H37Rv using the microplate Alamar blue (MABA) assay [12]. Only 29%, 49%, and 28% of inhibition of the mycobacteria was observed at 50 μg/mL of 1, 2, and 3, respectively. Few drimanes from medicinal *Warburgia* species [13-15] and the basidiomycete *Poria albocincta* BCC 26244 [16], and kauranes from the herb *Isodon rubescens* [17, 18] and the Vietnamese medicinal plant *Croton tonkinensis* [19], have been tested for their antimycobacterial potential.

In conclusion, a new drimane, 1-α-hydroxy-isodrimenine (1) and a new tetrahydroxy kauranol, 16-hydroxy-phlebia-*nor*-kauranol (2), along with the *N*-phenylacetamide (3), were isolated from the corticioid fungus *P. atropurpurascens* strain HM1. The structure of the new compounds was elucidated by spectroscopic and spectrometric methods, and their absolute configurations were unequivocally determined via single-crystal X-ray crystallography. Although drimanes and

kauranes are widely present in fungi, their biosynthesis, unique highly complex oxygenated skeletons, diverse biological activities, and drug-like chemical properties have attracted increasing attention. Even though only modest antimycobacterial activity was observed for the isolated compounds, this work represents a contribution to the knowledge of the secondary metabolism of a *Punctularia* strain isolated from Mexico.

Materials and Methods

General experimental procedures

Optical rotations, and UV data were measured using a Rudolph Research Autopol III polarimeter (Rudolph Research Analytical), and a Varian Cary 100 Bio UV-vis spectrophotometer (Varian Inc.), respectively. NMR spectra (1D and 2D NMR) were recorded on a Varian VNMRS 400 or 500 (400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C) or on a JEOL ECZ 600R (600 MHz for ^{1}H and 150 MHz for ^{13}C) in DMSO-d_6, methanol-d_4, or $CDCl_3$. Residual solvent signals were utilized for referencing were DMSO-d_6, δ_H/δ_C 2.50/39.5; methanol-d_4, δ_H/δ_C 4.78 and 3.31/49.2; and $CDCl_3$, δ_H/δ_C 7.24/77.2. HRMS data were acquired using a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific), equipped with an electrospray ionization source (ESI) with an HCD cell, in both positive and negative modes. LRMS data were acquired using a SQD2 mass spectrometer (Waters), equipped with an ESI, in both positive and negative modes. Samples were analyzed using an Acquity UPLC system (Waters) on a BEH C_{18} column (2.1 × 50mm, 1.7 μm) at 40°C, with a gradient system from 15:85 CH_3CN-0.1% aqueous formic acid to 100% of CH_3CN in 8min, then held for 1.5min with CH_3CN and returned to the starting conditions at a flow rate of 0.3mL/min. Analytical and preparative HPLC analyses were carried out on a Waters HPLC system equipped with PDA and ELSD detectors. Gemini C_{18} columns (Phenomenex) for analytical (4.6 × 250 mm, 5 μm) or preparative (21.2 × 250 mm, 5 μm) chromatography were employed. Data acquisition and management were performed with the Empower 3 software (Waters). Flash chromatography was conducted with a CombiFlash Rf+ Lumen system (Teledyne Technologies) equipped with PDA and ELSD detectors and using RediSep Rf Gold Si-gel columns (Teledyne Technologies). Reagent-grade chloroform, *n*-hexane, and methanol, and HPLC- and MS-grade acetonitrile, methanol, and water were purchased from J. T. Baker (Avantor). Deuterated NMR solvents were acquired from Cambridge Isotope Laboratories (Tewksbury).

Fungal material

The fungus *P. atropurpurascens* strain HM1 was isolated from a decorticated piece of *Quercus* bark collected in Bosque de Tlalpan, Mexico City, Mexico (2310 masl; 19°17′ 42.908″ N, 99°11′ 54.199″ W) in October of 2018. The axenic culture was kept at the personal fungal collection of the natural products research group at Facultad de Química, UNAM (voucher no. HM12018). Strain HM1 was identified using the internal transcribed spacer regions 1 & 2 and 5.8S nrDNA (ITS) using methods outlined previously with an ITS IF and ITS4 primer combination [20]. BLAST search results using the NCBI standard database indicated that ITS sequence from HM1 had ≥99% similarity with members of *Punctularia*. Based on the results of the BLAST search, maximum likelihood analysis was performed using *Punctularia* strains obtained from recent studies to identify strain HM1 using molecular phylogenetics [1,2]. All phylogenetic methods

were performed in PhyloSuite v1.2 [21]. Briefly, ModelFinder7 was used to select the best-fit model using Akaike Information Criterion; the best fitting substitution model according to AIC was SYM +I+G4. Maximum likelihood using IQTREE analysis revealed HM1 was sister to other strains of *P. atropurpurascens*, with strong Bayesian support (Supporting Information). Based on the BLAST search and maximum likelihood analysis with IQTREE, strain HM1 is identified as *Punctularia atropurpurascens*, Corticales, Punctulariaceae, Basidiomycota. The ITS sequence obtained in this study is deposited in GenBank under accession number OL840322.

Fermentation, extraction, and isolation

The isolate *P. atropurpurascens* strain HM1 was transferred to fresh PDA plates; subsequently, 1 cm agar plugs were added to 15 mL of YESD (soy peptone 2%, dextrose 2%, and yeast extract 1%) media and grown at room temperature for 7 days shaking at 100 rpm. Large-scale solid fermentations (10 × 250mL Erlenmeyer flasks containing 15 g of rice and 30 mL of H_2O) were inoculated with the seed cultures, incubated at room temperature for 21 days, and then extracted with 60 mL of MeOH-$CHCl_3$ (1 :1). Dried extracts were defatted by partition between a mixture of MeOH-CH_3CN (1:1) and hexane and evaporated to dryness. The extract (1.26 g) was fractionated via flash chromatography on RediSep Rf Gold Si-gel columns (40 g) using a gradient of *n*-hexane-$CHCl_3$-MeOH. This fractionation yielded 14 primary fractions: F1, 4.9 mg; F2, 42.0mg, F3, 517.9mg; F4, 278.9mg; F5, 9.5 mg; F6, 29.4mg; F7; 28.5; F8, 76.8mg; F9, 91.9mg; F10, 17.2mg; F11, 27.2mg; F12, 17.7mg; F13, 67.6mg; F14, 16.1 mg.

Fraction 6 was subjected to preparative HPLC. The mobile phase consisted of 0.1% aqueous formic acid (A) and CH_3CN (B). The following gradient was applied: 0 to 15 min: linear from 30% to 100% B. The flow rate was 21.24 mL/min. Column temperature: 40°C. This procedure yielded compounds 3 (2.9 mg, t_R = 7.50min) and 2 (1.1 mg, t_R = 17.5min). Fraction 11 was subjected to preparative HPLC using the same analytical conditions describe above, yielding 1 (4.3 mg, t_R = 14.5min).

Antibacterial activity

Pure compounds were tested against *M. tuberculosis* H37Rv strain (ATCC 27294) under aerobic (replicating) conditions using MABA [12]. Samples were dissolved in DMSO to obtain a stock solution and then tested at a final concentration of 50 μg/mL. The bioassays were carried out in 96-well plates in triplicate. The positive control was rifampicin (100% inhibition at 50 μg/mL).

X-Ray crystallography

Suitable X-ray quality single crystals for compounds 1 and 2 were obtained by recrystallization from methanol [22]. The crystallographic data and data collection parameters are presented in Table 2S (Supporting Information). All reflection intensities were measured at 100(2) K using a Gemini R diffractometer (equipped with Atlas detector) with CuKα radiation (λ = 1.54178 Å) under the program CrysAlisPro v1.171.38.43f (Rigaku OD, 2015). The most recent version of the program, v1.171.40.53 (Rigaku OD, 2019), was used for the refinement of cell dimensions and data reduction. The structure was solved with the program SHELXT-2018/2 and was refined on F2 by the full-matrix least-squares technique using SHELXL-2018/3 [23]. Analytical

absorption correction based on gaussian integration was applied using a multifaceted crystal model by CrysAlisPro. Non-hydrogen atoms were refined anisotropically. In the refinement, hydrogens attached to carbon were treated as riding atoms using SHELXL default parameters while those attached to oxygen were located with electron difference maps.

Isolates

1-*α-hydroxy-isodrimenine* (1): colorless crystals. α_D^{25} + 15 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (3.35) nm; ^1H (400 MHz) and ^{13}C (150MHz) NMR data: see Table 1. HRESIMS: m/z 251.1640 $[M + H]^+$ (calcd. for $C_{15}H_{23}O_3$, 251.1642, − 0.7 ppm).

16-*hydroxy-phlebia-nor-kauranol* (2): white crystals. [α_D^{25} + 26 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 200 (3.22) nm; 1H (500 MHz) and ^{13}C (125 MHz) NMR data: see Table 2. HRESIMS: m/z 339.2165 $[M + H]^+$ (calcd. for $C_{19}H_{31}O_5$, 339.2166, − 0.3 ppm).

Supporting Information

Molecular phylogenetic analysis of the fungal strain, MS and NMR data and UPLC profiles of 1-3, and X-ray crystallographic data of 1 and 2, are available as Supporting Information.

Contributors' Statement

Conceptualization and design: M. Figueroa; methodology: D. Acero, F. S. Tuglak Khan, A. J. Medina-Ortiz, L. Flores-Bocanegra, and B. Wan; formal analysis: D. Acero, H.A. Raja, C.A. Fajardo-Hernández, and M. Figueroa; writing original draft preparation, review, and editing: D. Acero, I. Rivero-Cruz, H.A. Raja, S.G. Franzblau, S. Hematian, and M. Figueroa. The manuscript was written and approved by all authors.

Acknowledgements

This work was supported by grants from UNAM DGAPA-PAPIIT IN222220 (M. F.) and FQ-PAIP 5000-9145 (M. F.). The authors are most thankful to Juan C. Rosas-Hernández and José A. Puerta Herrera from Facultad de Química, UNAM for their valuable help in the early stages of the project, and to Professor Nicholas H. Oberlies for providing access to the Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) facilities at the University of North Carolina at Greensboro. S.H. and F.S.T.K. gratefully acknowledge the Joint School of Nanoscience and Nanoengineering (JSNN), Greensboro, NC, for providing access to the X-ray diffraction facility.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Guan QH, Zhao W, Zhao ChL. A new species of Punctularia (Punctulariaceae, Basidiomycota) from southwest China. Phytotaxa 2021; 489: 285-292. doi:10.11646/phytotaxa.489.3.5
2. Knijn A, Saar I, Ferretti A. Biological characteristics of Punctularia atropurpurascens through morphological and molecular analyses during development. Ital J Mycol 2019; 48: 39-49. doi:10.6092/issn.2531-7342/9574
3. Ginns J. Genera of the North American Corticiaceae sensu lato. Mycologia 1998; 90: 1-35. doi:10.1080/00275514.1998.12026875
4. Anke H, Casser I, Steglich W, Pommer EH. Antibiotics from basidiomycetes. Phlebiakauranol aldehyde an antifungal and cytotoxic metabolite from Punctularia atropurpurascens. J Antibiotic 1987; 40: 443-449. doi:10.7164/antibiotics.40.443
5. Cali V, Spatafora C, Tringali C. Polyhydroxy-p-terphenyls and related pterphenylquinones from fungi: Overview and biological properties. Stud. Nat Prod Chem 2003; 29 (part J): 263-307. doi:10.1016/S1572-5995(03)80009-1
6. Aparicio-Cuevas MA, González MDC, Raja HA, Rivero-Cruza I, Kurina JS, Burdette JE, Oberlies NH, Figueroa M. Metabolites from the marine-facultative Aspergillus sp. MEXU 27854 and Gymnoascus hyalinosporus MEXU 29901 from Caleta Bay, Mexico. Tetrahedron Lett 2019; 60: 1649-1652. doi:10.1016/j.tetlet.2019.05.037
7. Maurs M, Azerad R, Cortés M, Aranda G, Bertranne-Delahaye M, Ricard L. Microbial hydroxylation of natural drimenic lactones. Phytochemistry 1999; 52: 291-296. doi:10.1016/s0031-9422(99)00199-5
8. Lisy JM, Clardy J. X-Ray structures of phlebiakauranol and phlebianorkauranol, highly oxygenated antibacterial metabolites of Phlebia strigosozonata. J Chem Soc Chem Commun 1975: 406-407. doi:10.1039/C39750000406
9. Chen S, Yong T, Xiao C, Su J, Zhang Y, Jiao C, Xie Y. Pyrrole alkaloids and ergosterols from Grifola frondosa exert anti-α-glucosidase and anti-proliferative activities. J Funct Foods 2018; 43: 196-205. doi:10.1016/j.jff.2018.02.007
10. Wu HH, Tian L, Chen G, Xu N, Wang YN, Sun S, Pei YH. Six compounds from marine fungus Y26-02. J Asian Nat Prod Res 2009; 11: 748-751. doi:10.1080/10286020903025783
11. Zou X, Liu S, Zheng Z, Zhang H, Chen X, Liu X, Li E. Two new imidazolone-containing alkaloids and further metabolites from the ascomycete fungus Tricladium sp. Chem Biodivers 2011; 8: 1914-1920. doi:10.1002/cbdv.201000372
12. Cho S, Lee HS, Franzblau S. Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA) for Mycobacterium tuberculosis. In: Parish T, Roberts DM, eds. Mycobacteria Protocols. New York, USA: Springer; 2015: 281-292

13. Wube AA, Bucar F, Gibbons S, Asres K. Sesquiterpenes from Warburgia ugandensis and their antimycobacterial activity. Phytochemistry 2005; 66: 2309-2315. doi:10.1016/j.phytochem.2005.07.018

14. Clarkson C, Madikane EV, Hansen SH, Smith PJ, Jaroszewski JW. HPLC-SPE-NMR characterization of sesquiterpenes in an antimycobacterial fraction from Warburgia salutaris. Planta Med 2007; 73: 578-584. doi:10.1055/s-2007-967187

15. Maroyi A. The genus Warburgia: A review of its traditional uses and pharmacology. Pharm Biol 2014; 52: 378-391. doi:10.3109/13880209.2013.837935

16. Isaka M, Chinthanom P, Danwisetkanjana K, Choeyklin R. A new cryptoporic acid derivative from cultures of the basidiomycete Poria albocincta BCC 26244. Phytochem Lett 2014; 7: 97-100. doi:10.1016/j.phytol.2013.10.009

17. Xu S, Li D, Pei L, Yao H, Wang C, Cai H, Yao H, Wu X, Xu J. Design, synthesis and antimycobacterial activity evaluation of natural oridonin derivatives. Bioorg Med Chem Lett 2014; 24: 2811-2814. doi:10.1016/j.bmcl.2014.04.119

18. Xu S, Pei L, Li D, Yao H, Cai H, Yao H, Wu X, Xu J. Synthesis and antimycobacterial evaluation of natural oridonin and its enmein-type derivatives. Fitoterapia 2014; 99: 300-306. doi:10.1016/j.fitote.2014.10.005

19. Jang WS, Jyoti MA, Kim S, Nam KW, Ha TKQ, Oh WK, Song HY. In vitro antituberculosis activity of diterpenoids from the Vietnamese medicinal plant Croton tonkinensis. J Nat Med 2016; 70: 127-132. doi:10.1007/s11418-015-0937-1

20. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: A primer for the natural products research community. J Nat Prod 2017; 80: 756-770. doi:10.1021/acs.jnatprod.6b01085

21. Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour 2020; 20: 348-435. doi:10.1111/1755-0998.13096

22. CCDC 2128612 (1) and 2128613 (2) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at: www.ccdc.cam.ac.uk/data_request/cif (accessed March 14, 2022), or by emailing data_request@ccdc.cam.ac.uk

23. Sheldrick GM. SHELXL-2018: Program for Crystal Structure Refinement. Göttingen, Germany: University of Göttingen; 2018. Accessed March 14, 2022 at: www.shelx.uni-goettingen.de/