Tetrahedron Letters 60 (2019) 151157

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Talarophenol sulfate and talarophilones from the Australian mud dauber wasp-associated fungus, *Talaromyces* sp. CMB-W045

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ARTICLE INFO

Article history:
Received 25 August 2019
Revised 10 September 2019
Accepted 15 September 2019
Available online 16 September 2019

Keywords:
Talaromyces
Talarophenol
Talarophilones
Antibacterial

ABSTRACT

Chemical analysis of a jasmine rice cultivation of an Australian mud dauber wasp-associated fungus, *Talaromyces* sp. CMB-W045, led to the discovery of a new *p*-terphenyl, talarophenol sulfate (**1**). The structure elucidation of **1** was achieved by detailed spectroscopic analysis supported by acid hydrolysis to the *p*-hydroquinone talarophenol (**2**), and subsequent *in situ* air oxidation to trace amounts of the *p*-quinone talaroquinone (**3**). The same jasmine rice cultivation also yielded the new talarophilones A (**4**) and B (**5**), and known (+)-mitorubrin (**6**) and pochonin D (**7**), with structures assigned by detailed spectroscopic analysis. Neither **1** or **4**–**7** exhibited growth inhibitory properties against a panel of human cell lines, or bacterial or fungal pathogens, although **1** did exhibit selective antibacterial activity against *Streptococcus pyogenes* ATCC 12344 (IC_{50} 10 μM).

Introduction

In earlier studies [1,2] we reported on the secondary metabolites produced by fungi isolated from an Australian mud dauber wasp. These included an account of an unprecedented nitro *depsi*-tetrapeptide diketopiperazine, waspergillamide A, produced by a jasmine rice cultivation of *Aspergillus* sp. CMB-W031 [2]. We also explored the differing chemistry encountered when *Talaromyces* sp. CMB-W045 was cultivated on jasmine versus red rice, revealing the importance of Fe(III) levels, and leading to the discovery of a suite of new fungal siderophores, talarazines A-E [1]. This current study describes an investigation into the non-siderophore natural products produced by jasmine rice cultivations of *Talaromyces* sp. CMB-W045, leading to the isolation and structure elucidation of the first reported example of a sulfated *p*-terphenyl natural product, talarophenol sulfate (**1**), as well as the new azaphilones, talarophilones A (**4**) and B (**5**), and known analogues, (+)-mitorubrin (**6**) [3–5] and pochonin D (**7**) [6]. Structure elucidation was achieved by detailed spectroscopic analysis, supported by acid hydrolysis of **1** to yield talarophenol (**2**), which underwent partial air oxidation to talaroquinone (**3**). The production, isolation, characterization, structure elucidation and biological profiling of **1**–**7** is summarized below.

Results and discussion

An assessment of the relationship between *Talaromyces* sp. CMB-W045 cultivation conditions versus secondary metabolite production was carried out in a 24-well plate microbioreactor format (MATRIX) using multiple media compositions, under solid agar and grain, as well as shaken and static broth conditions [7].

Following *in situ* solvent extraction, the resulting extracts (×38) were subjected to chemical profiling (HPLC-DAD-ESIMS). Of note, jasmine rice solid cultivation revealed a particularly promising secondary metabolite profile. Subsequent scaled up cultivation and extraction (40 × 250 mL flasks, each charged with 20 g of jasmine rice), followed by solvent partitioning and trituration, and SPE and HPLC fractionation, yielded **1** and **4**–**7**. While comparison of experimental with literature spectroscopic data identified the known fungal azophilones, (+)-mitorubrin (**6**) [3–5] and pochonin D (**7**) [6], the structures of **1** and **3**–**5** were identified by detailed spectroscopic analysis, as summarised below.

HRESI(−)MS analysis of **1** returned a *quasi*-molecular ion ($[M-H]^-$) consistent with a molecular formula ($C_{20}H_{17}O_8S$, Δppm − 1.9) suggestive of a highly oxidized polyaromatic compound. The ^{13}C NMR (DMSO d_6) data for **1** (Tables 1 and S1) revealed × 15 carbon resonances, further resolved to × 16 resonances when the data was re-acquired in methanol d_4 (Figure S5), supportive of symmetry. Further analysis of the 1D NMR (DMSO d_6) data for **1**

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https://doi.org/10.1016/j.tetlet.2019.151157
0040-4039/

attributed this symmetry to two *p*-substituted phenoxy moieties (*ring A*: C-1 to C-6; δ_H 7.29, 6.76; δ_C 124.1, 131.6, 114.3, 156.1; and *ring C*: C-1″ to C-6″; δ_H 7.48, 6.99; δ_C 130.6, 130.0, 113.6, 158.1), with diagnostic 2D NMR (DMSO d_6) correlations establishing *ring A* as a *p*-substituted phenol, and *ring C* as a *p*-substituted anisole (Figure 2).

Further analysis of the 1D NMR (DMSO d_6) data for **1** required that the remaining structural fragment ($C_7H_5O_6S$) be a penta-substituted benzene (*ring B*: C-1′ to C-6′; δ_H 7.34; δ_C 126.1, 117.2, 143.3, 126.1, 145.5, 142.8) bearing phenol (δ_H 8.44), methoxy (δ_H 3.23; δ_C 59.7) and sulfate residues, as well as *ring A* and *ring C*. Analysis of the 2D NMR data for **1** in both DMSO d_6 (Table S1 and Figures 2 and S4, S6-S10) and methanol d_4 (Figures 2 and S5) provided data to assign the *ring B* regiochemistry. Diagnostic HMBC (DMSO d_6) correlations from 5′-OC<u>H</u>$_3$ (δ_H 3.23) to C-5′ (δ_C 145.5), and from 6′-O<u>H</u> (δ_H 8.44) to both C-6′ (δ_C 142.8) and C-5′, required that the OCH_3 and OH substituents to *ring B* were *ortho* disposed, further confirmed by a ROESY (DMSO d_6) correlation between 5′-OCH_3 and 6′-OH. In light of the above, an HMBC (DMSO d_6) correlation from 6′-OH to the overlapping C-1′/C-4′ resonance (δ_C 126.1) supported an *ortho* disposition between 6′-OH and C-1′. Better dispersion of the C-1′ and C-4′ resonances in methanol d_4 revealed HMBC correlations from H-2/H-6 (δ_H 7.39) to C-4′ (δ_C 128.6), and from H-2″/H-6″ (δ_H 7.60) to C-1′ (δ_C 128.3), consistent with *ring A* and *ring C* substituents at C-4′ and C-1′, respectively. Similarly, an HMBC (methanol d_4) correlation from H-2′ (δ_H 7.43) to C-1″ required that H-2′ be *ortho* to C-1′ (and the *ring C* substituent), and a ROESY (DMSO d_6) correlation between 5′-OCH_3 and H-2/H-6 required that 5′-OCH_3 be *ortho* to C-4′ (and the *ring A* substituent). By default, the regiochemistry of the putative *ring B* sulfate residue was as indicated (Figure 1).

To further validate the presence and regiochemistry of a *ring B* sulfate moiety in **1**, we turned to chemical degradation (Figure 3). Acid hydrolysis of an aliquot of **1** (0.1% TFA in MeCN at r.t. overnight) effected quantitative conversion to the anticipated talarophenol (**2**) ($C_{20}H_{18}O_5$, Δppm − 0.4) and trace levels of talaroquinone (**3**) ($C_{20}H_{16}O_5$, Δppm − 0.2) (Figure S11). Comparison of ^{1}H NMR (DMSO d_6) data for **2** with **1** (Table 1) revealed excellent concordance, with the only significant difference being a substantial shielding of the H-2′ resonance in **2** compared to **1** ($\Delta\delta_H$ + 0.77), consistent with an *ortho* relationship between H-2′ and the sulfate substituent in **1**.

HRESI(+)MS analysis of **4** returned a pseudo-molecular ion ([M + Na]$^+$) indicative of a molecular formula ($C_{21}H_{20}O_7$, Δppm 3.6) requiring × 12 DBE. Similarities in the 1D NMR (DMSO d_6) data for **4** and the known azaphilone **6** (Tables 2 and S3 and S5) suggested that **4** is a $\Delta^{3,4}$ reduced analogue of **6**. Key NMR differences supportive of this hypothesis were replacement of the H-4 sp^2 methine in **6** (δ_H 6.61, s) with a diastereotropic H_2-4 sp^3 (H-4α, δ_H 2.99; H-4β, δ_H 2.90) coupled to an H-3 sp^3 oxymethine (H-3, δ_H 4.96). The 2D NMR (DMSO d_6) data for **4** (Table S3 and Figure 4) revealed correlations attributed to an orsellinic acid moiety (C-1″ to C-7″) attached via an ester linkage to a bicyclic azaphilone scaffold, permitting assembly of the planar structure for talarophilone A (**4**). As **4** is a co-metabolite with (+)-mitorubrin (**6**) of known absolute configuration [3–5], on biosynthetic grounds we tentatively assign a common 7*S* configuration, although the C-3 configuration remains unassigned.

HRESI(+)MS analysis of **5** returned a pseudo-molecular ion ([M + Na]$^+$) indicative of a molecular formula ($C_{23}H_{26}O_8$, Δppm −0.22) also suggestive of an azaphilone. Comparison of the 1D NMR ($CDCl_3$) data for **5** (Tables 2 and S22-23) with **4** revealed a common orsellinic ester moiety, and H_2-4 sp^3 methylene (H-4α, δ_H 2.35; H-4β, δ_H 2.21) coupled to an H-3 sp^3 methine (δ_H 4.06).

Key differences in the NMR data centered around replacement of the H-1 and H-5 sp^2 methines, $\Delta^{1,8a}$ and $\Delta^{4a,5}$, and C-6 ketone carbonyl in **4**, with diastereotopic H_2-1 (H-1α, δ_H 4.57; H-1β, δ_H 4.30) and H_2-5 (H-5α, δ_H 2.69; H-5β, δ_H 2.54) methylenes, and a $\Delta^{4a,8a}$ (δ_C 149.6, 130.4) and 6-OAc (δ_H 2.06; δ_C 170.1, 21.1) in **5**. Diagnostic 2D NMR correlations (Figure 4) permitted assembly of

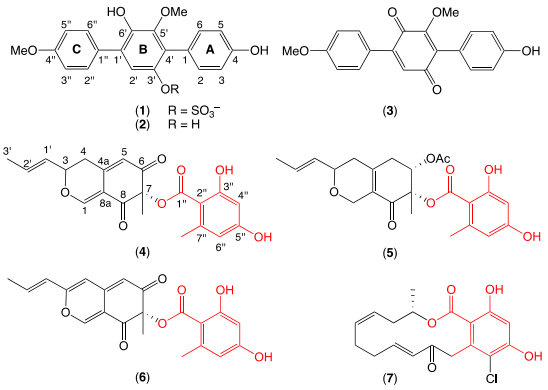

Figure 1. *Talaromyces* sp. CMB-W045 associated chemistry **1**–**7.**

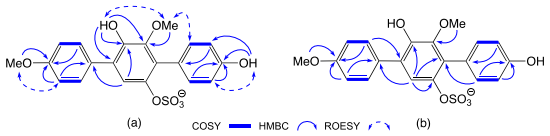

Figure 2. Diagnostic 2D NMR correlations for **1** in (a) DMSO d_6 and (b) methanol d_4.

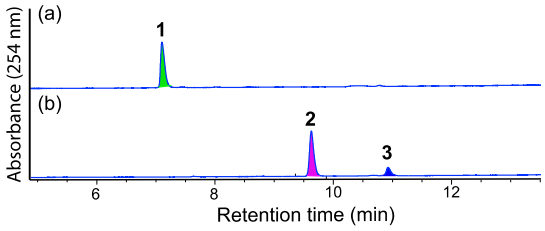

Figure 3. HPLC-DAD (254 nm) chromatograms of (a) **1** and (b) the product from acid hydrolysis of **1** with 0.1% TFA in MeCN.

Table 1
1D NMR (600 MHz, DMSO d_6) data for compounds **1**–**2.**

1			**2**
Position	δ_C	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1	124.1		
2/6	131.6	7.29, d *(8.4)*	7.18, d *(8.5)*
3/5	114.3	6.76, d *(8.4)*	6.78, d *(8.5)*
4	156.1		
4-OH		9.35, s	9.37, s
1′	126.1[a]		
2′	117.2	7.34, s	6.57, s
3′	143.3		
3′-OSO_3^-			
3′-OH			8.02, s
4′	126.1[a]		
5′	145.5		
5′-OMe	59.7	3.23, s	3.26, s
6′	142.8		
6′-OH		8.44, s	8.65, s
1″	130.6		
2″/6″	130.0	7.48, d *(8.6)*	7.47, d *(8.6)*
3″/5″	113.6	6.99, d *(8.6)*	6.97, d *(8.6)*
4″	158.1		
4″-OMe	55.1	3.79, s	3.78, s

[a] Overlapping resonances

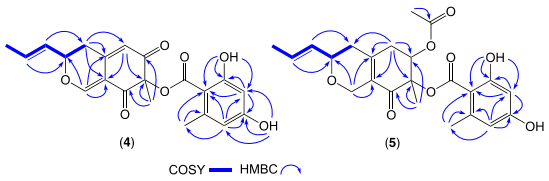

Figure 4. Diagnostic 2D NMR correlations for **4** in DMSO d_6 and **5** in $CDCl_3$.

the planar structure for talarophilone B (**5**) which, following the biosynthetic argument presented above, was tentatively assigned a 7*S* configuration in common with **6**.

Talarophenol sulfate (**1**), and talarophilones A (**4**) and B (**5**), did not exhibit growth inhibitory activity against a panel of human cancer cell lines, or bacterial and fungal pathogens, but **1** did exhibit modest antibacterial activity (IC_{50} 10 μM) against *Streptococcus pyogenes* ATCC 12344.

Conclusion

One of the earliest fungal *p*-terphenyl reported was a 1975 account of terphenyllin from *Aspergillus candidus* ATCC 20022 [8]. Since then additional fungal *p*-terphenyls have been reported, with some exhibiting biological properties inclusive of antifungal [9], cytotoxic [10,11], α-glucosidase inhibitory [12] and acetylcholinesterase activity [11]. Notwithstanding these past accounts, to the best of our knowledge talarophenol sulfate (**1**) is the first example of a sulfated *p*-terphenyl. The absence of such sulfates in the scientific literature may reflect a bias among natural products chemists in favor of extraction, fractionation and/or handling in acidic media and/or solvents (*i.e.*, silica and $CHCl_3$, and acid HPLC modifiers), leading to *in situ* hydrolysis of any sulfate moieties.

Likewise, azaphilones are a structurally diverse family of fungal polyketides possessing a highly oxygenated pyranoquinone bicyclic core, usually known as an isochromene [13]. Known examples can be classified into many structural groups, including citrinins, spiciferinones, austdiols, helotialins, deflectins, bulgarialactones, sequoiatones, trichoflectins, ascochitines, chaetoviridins, chaetomugilins, multiformins, cohaerins, spiroazaphilones, chlorofusins, nitrogenated azaphilones and mitorubrins. The azaphilones have attracted a great deal of interest owing to their diverse biological activities and structural features, with over × 170 reported from × 23 genera of fungi [14]. The discovery of talarophilones A (**4**) and B (**5**) adds to this diversity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank A. Rolland for isolating fungal strain CMB-W045, M. Quezada for fungal taxonomy and for performing cytotoxicity assays on talarophilones A and B and mitorubrin, and E. Lacey for assistance in fungal cultivation. PK acknowledges the University of Queensland for an international postgraduate scholarship. This research was funded in part by the Institute for Molecular Bioscience, the University of Queensland and the Australian Research Council (DP120100183).

Table 2
1D NMR (600 MHz) data for compounds **4** and **5.**

4[a]			**5**[b]	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	161.5	8.03, s		
1α			63.6	4.57, br d *(16.2)*
1β				4.30, br d *(16.2)*
3	78.5	4.96, ddd *(9.8, 6.9, 4.0)*	73.6	4.06, m
4α	32.4	2.99, dd *(17.0, 4.0)*	36.6	2.35, dd *(18.5, 10.4)*
4β		2.90, dd *(17.0, 9.8)*		2.21, ddd *(18.5, 3.0, 3.0)*
4a	147.6		149.6	
5	115.9	5.90, s		
5α			35.0	2.69, dd *(17.1, 6.0)*
5β				2.54 dd *(17.1, 11.5)*
6	191.0		69.8	6.17, dd *(11.5, 6.0)*
6-$\underline{C}OCH_3$			170.1[c]	
6-CO$\underline{CH_3}$			21.1	2.06, s
7	84.9		83.6	
7-Me	22.2	1.53, s	17.3	1.54, s
8	192.7		191.6	
8a	110.4		130.4	
1′	127.2	5.67, ddq (*15.1, 6.9, 1.3*)	130.3	5.52, ddq *(15.3, 6.4, 1.5)*
2′	131.6	5.95, dq *(15.1, 6.4)*	129.3	5.79, dq *(15.3, 6.4)*
3′	17.5	1.73, br d *(6.4)*	18.1	1.72, br d *(6.4)*
1″	168.1		170.0[c]	
2″	104.6		105.9	
3″	162.9		165.7	
3″–OH		10.38, s		11.0, s
4″	100.6	6.16, d *(2.0)*	101.6	6.21, d *(2.3)*
5″	162.4		160.6	
5″–OH		10.29, s		[d]
6″	111.2	6.25, d *(2.0)*	111.6	6.18, d *(2.4)*
7″	142.5		144.1	
7″-Me	22.7	2.46, s	24.3	2.46, s

[a]DMSO-d_6, [b] MeOH d_4, [c] assignments are interchangeable, [d] resonance is not observed

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151157.

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