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Meroterpenoids with Antiproliferative Activity from a Hawaiian-Plant Associated Fungus *Peyronellaea coffeae-arabicae* FT238

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S *Supporting Information*

ABSTRACT: Three unusual polyketide-sesquiterpene metabolites peyronellins A–C (**1**–**3**), along with the new epoxyphomalin analog 11-dehydroxy epoxyphomalin A (**4**), have been isolated from the endophytic fungus *Peyronellaea coffeae-arabicae* FT238, which was isolated from the native Hawaiian plant *Pritchardia lowreyana*. The structures of compounds **1**–**4** were characterized based on NMR and MS spectroscopic analysis. The absolute configuration (AC) of the compounds was determined by electronic circular dichroism (ECD). Compound **4** showed antiproliferative activity with an IC_{50} of 0.5 μM against OVCAR3, and it also strongly inhibited Stat3 at 5 μM.

Fungi have been an important source of valuable and novel small molecules for drug development.[1,2] Endophytic fungi, which usually live asymptomatically within the tissues of higher plants, are known to produce structurally unique and biologically active secondary metabolites.[3,4] During an ongoing search for new and bioactive compounds from filamentous fungi collected in Hawai'i, about 5000 semipure fractions from fungal culture extracts were screened against cisplatin-sensitive A2780 (A2780, human ovarian cancer) and cisplatin-resistant A2780 (A2780cisR, Stat3 abnormally activated) cell lines, together with OVCAR3. The results showed that one semipure fraction produced by an endophytic fungus *Peyronellaea coffeae-arabicae* FT238 was active against A2780, A2780cisR, and OVCAR3 at 20 μg/mL. Bioassay guided separation of the active fraction led to the isolation of four new terpene-polyketide hybrid metabolites **1**–**4** (Figure 1), of which **4** showed Stat3 inhibitory activity and was active against OVCAR3 with an IC_{50} value of 0.5 μM.

Compound **1**[5] was isolated as a yellowish solid. Its molecular formula was determined to be $C_{28}H_{36}O_7$ by HR-ESIMS (m/z 485.2517, calcd for $[M + H]^+$ 485.2539), with 11 degrees of unsaturation. The IR spectrum showed the existence of hydroxyl (3379 cm^{-1}) and carbonyl (1659 cm^{-1}) groups. A detailed analysis of ^1H and ^{13}C NMR spectra (Table 1) demonstrated the presence of four methyl signals, seven methylenes with one oxygenated (δ_H 3.29/3.00, δ_C 71.5), seven methines including three olefinic and two oxygenated, and ten other carbons with no hydrogen attached, seven of which are sp^2 hybridized. One α,β-unsaturated ketone (δ_C 197.4), four double bonds, one carbonyl group (δ_C 178.4), and one epoxide (δ_H 3.26/δ_C 57.9, δ_C 65.9), accounting for seven degrees of unsaturation, were determined to be present in the molecule. The remaining four degrees of unsaturation suggested the existence of four rings. The planar structure of compound **1** was elucidated on the basis of 2D NMR spectra including COSY, HSQC, and HMBC. The observed HMBC correlations from H-5 to C-3 and C-4; from H_2-2 and H_2-6 to C-4; from H_2-2 to C-10; from H_3-13 to C-1, C-5, C-9; from H_2-15 to C-9 and C-10; from H_3-14 to C-7 and C-9; and from H_3-12 and H_2-11 to C-3, C-4, and C-5 established a sesquiterpene moiety as shown in Figure 2 (red part). In the polyketide moiety, ring C was established on the basis of the main HMBC correlations from H-2′ (δ_H 3.26) to C-3′ (δ_C 197.4) and C-4′ (δ_C 135.0); from H-5′ (δ_H 6.11) to C-1′; and also from

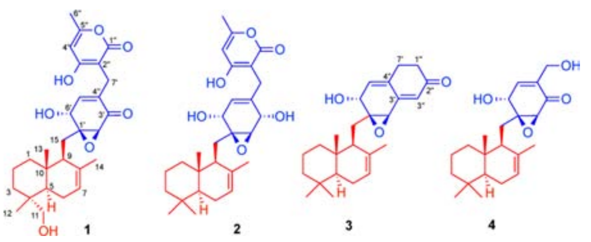

Figure 1. Structures of compounds **1**–**4**.

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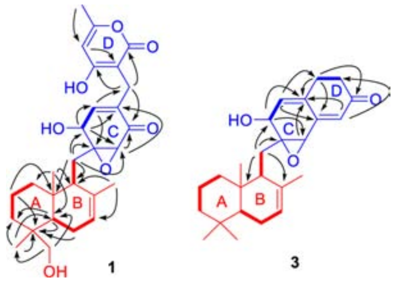
Table 1. NMR Spectroscopic Data for 1 and 2 in MeOH-d_4

	1		**2**	
no.	δ_H, J (Hz)[a]	δ_C[b]	δ_H, J (Hz)[a]	δ_C[b]
1α	0.91, br. dd, 13.1, 3.1	40.0	0.91, m	40.4
1β	1.80, br. d, 13.1		1.82, m	
2α	1.60, br. s	18.9	1.56, m	19.5
2β	1.53, m		1.46, m	
3α	1.48, m	36.6	1.43, m	43.3
3β	1.27, m		1.20, m	
4		38.4		33.5
5	1.56, m	44.1	1.22, m	51.3
6	1.92−1.80, m	24.3	2.00−1.92, m	24.6
7	5.39, br. s	122.8	5.38, dd, 5, 1.5	122.7
8		135.6		136.2
9	1.58, br. d, 7.9	47.6	1.57, m	47.8
10		36.1		37.0
11	3.29, d, 10.9	71.5	0.86, s	33.4
	3.00, d, 10.9			
12	0.82, s	17.9	0.89, s	22.0
13	0.84 s	14.5	0.79, s	14.0
14	1.64, s	21.9	1.65, s	22.2
15α	2.21, d, 15.9	26.3	2.11, d, 16.0	27.0
15β	2.02, dd, 15.9, 7.9		1,80, dd, 16.0, 7.9	
1′		65.9		61.6
2′	3.26, s	57.9	3.25, d, 2.1	58.1
3′		197.4	4.41, br. s	67.2
4′		135.0		136.8
5′	6.11, d, 4.6	139.7	5.38, dd, 4.7, 1.3	122.9
6′	4.33, d, 4.6	67.6	4.09, d, 4.7	68.1
7′	3.37, d, 17.2	22.9	3.20, br. s	26.6
	3.05, d, 17.2			
1″		178.4		172.4
2″		96.4		99.6
3″		169.8		169.2
4″	5.80, s	107.0	5.95, s	103.6
5″		160.6		161.6
6″	2.14, s	19.3	2.18, s	19.3

[a]Spectra recorded at 500 MHz. [b]Spectra recorded at 125 MHz. Data based on ^1H, ^{13}C, HSQC, and HMBC experiments.



1 3

Figure 2. $^1H-^1H$ COSY (bolds) and selected HMBC (arrows) correlations of compounds **1** and **3**.

H-6′ (δ_H 4.33) to C-1′, C-4′, and C-5′. The observed HMBC correlations from H_2-15 to C-1′, C-2′, and C-6′ indicated that ring C was connected at C-1′ with ring B through C-15. Besides the established rings A−C and the substituents, the remaining $C_7H_7O_3$ was proposed to form a 3-substituted 4-hydroxy-6-methyl-2*H*-pyran-2-one moiety. This deduction was verified by the observed HMBC correlations from H-4″ to C-2″ and C-6″; from H_3-6″ to C-4″ and C-5″; and from H_2-7′ to C-1″ and C-3″. Furthermore, rings C and D were suggested to be connected to each other through C-7′ due to the HMBC correlations from H-7′ to the carbons in both rings C and D as shown in Figure 2. Hence, the planar structure of **1** was determined. A literature search suggested that the moiety of rings A−C was very similar to those of the potent cytotoxic fungal metabolites epoxyphomalins A and B.[6] The relative configuration of compound **1** was determined to be the same as that of epoxyphomalins A and B by the detailed analysis of the ROESY correlations as shown in Figure S1.

Compound **2**[7] was isolated as a yellowish solid. Its molecular formula was determined to be $C_{28}H_{38}O_6$ by HR-ESIMS (m/z 493.2546, calcd for $[M + Na]^+$ 493.2566), with 10 degrees of unsaturation. The IR spectrum showed the existence of hydroxyl (3352 cm^{-1}) and carbonyl (1660 cm^{-1}) groups. Detailed comparison of the NMR data of **2** (Table 1) with those of **1** showed that both were very similar, except that the *α*,*β*-unsaturated ketone and hydroxylated methylene in the molecule of **1** were absent in that of **2**. Meanwhile, one more hydroxylated methine (δ_H 4.41/δ_C 67.2) and one more methyl group were present in **2**. The HMBC correlations from the hydroxylated methine H-3′ to C-1′, C-5′, and C-7′ in **2** suggested that the ketone in compound **1** was reduced to a hydroxyl group in **2**, which was also supported by the upfield shift of the olefinic proton H-5′ from δ_H 6.11 in **1** to δ_H 5.38 in **2**. The hydroxylated methylene in **1** was replaced by a methyl group in **2** based on the HMBC correlations from the methyl group (CH_3-11) to C-3, C-4, and C-5. The relative configuration of **2** except 3′ was determined to be the same as that of **1** by analysis of ROESY data (Figure S1). *J*-values between H-2′ and H-3′ with a *cis*- and *trans*-relationship between the epoxide and the 3′-hydroxyl were reported,[8] but not diagnostic.

Compound **3**[9] was isolated as a yellowish solid. HR-ESIMS gave a quasi-molecular ion at m/z 383.2580, suggesting a molecular formula of $C_{25}H_{34}O_3$ (calcd for $[M + H]^+$ 383.2581), with 9 degrees of unsaturation. Comprehensive analysis of the NMR data (Table 2) including ^1H, ^{13}C, HSQC, and HMBC indicated the presence of four methyls, seven methylenes, seven methines (three olefinic), and seven other carbons with no hydrogen attached, among which three were olefinic carbons and one was an *α*,*β*-unsaturated ketone. Detailed comparison of the NMR data of **3** with those of **2** showed that both had the same sesquiterpene moiety, as demonstrated by the COSY and HMBC correlations shown in Figure 2. Ring C was also determined to be present in **3** based on the HMBC correlations from the oxygenated proton H-6′ to C-1′ and C-2′ (epoxide carbons), and C-4′ and C-5′, and from the olefinic proton H-5′ to C-1′ and C-3′. Since the three double bonds, the one *α*,*β*-unsaturated ketone, three rings A−C, and the one epoxide moiety accounted for eight degrees of unsaturation, the remaining degree of unsaturation suggested the presence of one more ring in **3**. The COSY correlation between H-7′ and H-1″ and HMBC correlations from the olefinic proton H-3″ to C-2′, C-4′, and C-1″; from H-1″ to C-4′ and C-3″; and from H-7′ to the ketone C-2″ suggested the presence of a six-membered ring D with an *α*,*β*-unsaturated ketone moiety. The relative configuration of the sesquiterpene moiety was presumed to be the same as that of compound **1** by the analysis of key ROESY correlations (Figure S1; Table S1).

Compound **4**[10] was isolated as a yellowish powder. Its molecular formula was determined to be $C_{22}H_{32}O_4$ by HR-ESIMS (m/z 361.2389, calcd for $[M + H]^+$ 361.2373). The NMR spectra (Table 2) including ^1H, ^{13}C, and HSQC demonstrated the presence of 22 carbons including four methyls, six

Table 2. NMR Spectroscopic Data for 3 and 4 in MeOH-d_4

	3		**4**	
no.	δ_H, J (Hz)[a]	δ_C[b]	δ_H, J (Hz)[a]	δ_C[b]
1α	0.98, br. dd, 13.1	40.4	0.91, m	40.6
1β	3.2, 1.80, m		1.80, m	
2α	1.58, m	19.8	1.60, m	19.8
2β	1.46, m		1.44, m	
3α	1.43, m	43.4	1.44, m	43.4
3β	1.23, m		1.22, m	
4		33.7		33.9
5	1.25, m	51.4	1.22, m	51.4
6α	1.98, m	24.9	1.98, m	24.8
6β	1.88, m		1.88, m	
7	5.41, br. s	123.1	5.42, br. s	123.3
8		136.1		135.8
9	1.64, m	48.1	1.54, m	48.1
10		37.3		37.3
11	0.87, s	33.7	0.87, s	33.7
12	0.90, s	22.3	0.90, s	22.3
13	0.82 s	14.3	0.82, s	14.3
14	1.66, s	22.3	1.68, br. s	22.2
15α	2.19, dd, 16.1, 1.9	27.1	2.23, dd, 16.1, 1.9	26.7
15β	1.95, dd, 16.1, 8.1		2.05, dd, 16.1, 8.1	
1′		63.7		66.3
2′	3.56, d, 1.4	56.8	3.25, br. s	58.0
3′		153.1		196.6
4′		131.2		136.7
5′	5.94, d, 5.2	129.6	6.68, dt, 5.0, 1.8	141.1
6′	4.38, d, 5.3	68.5	4.49, d, 5.0	67.4
7′	2.67, m	30.5	4.24, dd, 15.1, 0.9	59.4
			4.19, dt, 15.1, 1.5	
1″	2.52, m	38.3		
2″		200.9		
3″	6.22, d, 1.6	127.5		

[a]Spectra recorded at 500 MHz. [b]Spectra recorded at 125 MHz. Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments.

methylenes including one oxygenated, six methines including two olefinic and two oxygenated, and six other carbons with no hydrogen attached, one of which is an α,β-unsaturated ketone (δ_C 196.6). A detailed analysis of the 1D and 2D NMR data showed that compound **4** had the same skeleton as that of epoxyphomalin A.[6] However, there were two hydroxylated methylenes and three methyl groups in epoxyphomalin A, but only one hydroxylated methylene and four methyl groups in **4**. This observation implied that one hydroxylated methylene at the 11-position in epoxyphomalin A was reduced to a methyl in **4**. The HMBC correlations of the methyl H$_3$-11 with C-3, C-4, C-5, and C-12 in **4** confirmed the above deduction.

Since **1** had the same CD spectral pattern (Figure S2) as that of epoxyphomalin A,[6] the AC of compound **1** should be the same as that of epoxyphomalin A, which was supported by the calculated ECD spectrum of epoxyphomalin A (Figure S3). The positive Cotton effect (CE) at 220 nm and the negative CEs at 250 and 340 nm of epoxyphomalin A were theoretically reproduced at 230, 280, and 350 nm, respectively. The CD spectrum of compound **2** (Figure S5), an analog of **1** and craterellin A,[11] implied that the ACs at C5, C9, C10, C1′, C2′, and C6′ must be the same as that of compound **1**, leaving only that at C3′ unassigned (**2a** and **2b** in Figure S6). The J-value between H-2′ and H-3′ was calculated as 3.71 Hz for the 3′*R*-configuration (**2a**) but 2.11 Hz for the 3′*S*-**2** (**2b**), matching well with the experimentally observed value of 2.1 Hz. Surprisingly, neither the weighted ECD spectra of **2a** nor those of **2b** match the experimental CD curve (Figure S7). However, careful observation of the ECDs of their individual conformers indicates that none of the calculated ECDs of individual conformers of **2a** matches the experimental CD but some minor conformers of **2b** do, implying that **2b** must be the AC of **2** but **2a** cannot be (see Figure 3 and Supporting Information (SI) for detailed analysis).

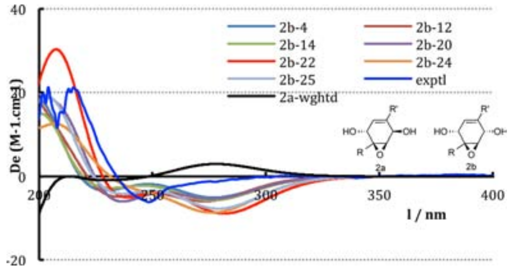


Figure 3. Experimental CD, weighted ECD of **2a**, and computed ECD of some individual conformers of absolute configuration **2b** of compound **2**.

Hence, only the *S*-configuration at 3′, instead of the *R*-, was supported by the calculated ECD of **2** (see Figure S7, Tables S3 and S4 for computation information). ECD was also computed to assign the AC of **3**. Out of eight possible structures (see Figure S8), only **3a** and **3b** were selected for the ECD computation (see SI for detailed information). Among the four new compounds, only **3** had positive CEs at 270 and 350 nm (Figure S9), so we took it for granted that the AC of **3** should be **3a** in which the ACs at C1′, C2′, and C6′ are enantiomeric to those in **1**. However, the weighted ECD of **3b** was found to match the experimental CD curve very well (Figure 4), to which that of **3a** was found opposite. Hence, the AC of **3** was assigned as 5*S*,9*S*,10*S*,1′*S*,2′*R*,6′*R* (**3b**) (Figure 1). The different conjugated system (−CH═CR−CR═CH−CO−R) could help explain why compound **3** shares the same C-ring AC with **1** but produces opposite Cotton effects. A slight change in the structure can generate a totally different CD spectrum, as exemplified by the case of rhodonoids A and B.[12] From a biosynthetic point of view, compound **4**, 11-dehydroxy epoxyphomalin A, should also have the same AC as that of epoxyphomalin A,[6] which was supported by analogous CD cotton effects (Figure S12). All computations at the quantum mechanics levels were carried out using the Gaussian 09 software.[13]

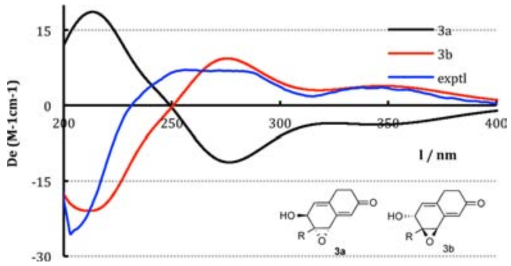
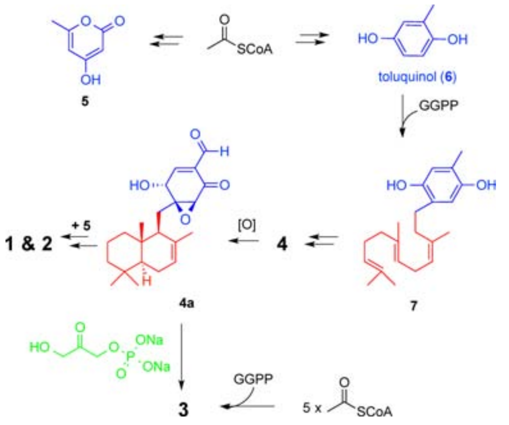


Figure 4. Experimental CD and computed ECD of possible absolute configurations of compound **3**.

The biosynthesis of the isolated compounds **1**−**4** was proposed as shown in Scheme 1. All four compounds seem to be biogenetically related to other meroterpenoids, such as arisugacin from *Penicillium* sp. FO-4259,[14] territrem C from

Scheme 1. Proposed Biosynthetic Pathways for Compounds 1−4



Aspergillus terreus,[15] pyripyropenes from *Aspergillus fumigatus*,[16] yaminterritrems A and B from *Aspergillus terreus*,[17] actinoranone from a *Streptomyces* sp.,[18] and epoxyphomalins from *Paraconiothyrium* sp.[19] Coupling of **6**[20] with GGPP[20] could yield 11-dehydroxy epoxyphomalin A (**4**) via **7**.[20] **4** could also be formed through the coupling between a cyclized sesquiterpene with an electron-rich vinyl moiety and an enone form of **6**, followed by epoxidation. Peyronellins A and B (**1** and **2**) might be derived from **4a** and the δ-lactone (4-hydroxy-6-methyl-2*H*-pyran-2-one) (**5**). Conjugation of the oxidized product **4a** with dihydroxyacetone phosphate[21] could afford peyronellin C (**3**), which could also be produced directly from a sesquiterpene and a polyketide derived tetrahydronaphthalenone.

Compounds **1**−**4** were tested against a panel of cancer cell lines, and only compound **4** was active at low μM or high nM levels (Table 3). Compound **4** was 3- to 4-fold more potent against OVCAR3 (mt-p53^{R248}) than the other tested cancer cell lines except A2780 and A2780 CisR. Compound **1** was also active against A2780 and A2780 CisR (Table 3), but **2** and **3** were inactive. As a master regulator, Stat3 is constitutively activated in many types of cancer and it affects many important processes of cancer biology. Since **4** could inhibit A2780 CisR, a cancer cell line with Stat3 abnormally activated, at 0.6 μM, we evaluated the inhibitory activity of **4** at 5 μM against Stat3 with MDA-MB-231 breast cancer cells. The results showed that **4** inhibited intracellular phosphor-tyrosine Stat3 (pY705Stat3), suggesting the potential inhibition of aberrant Stat3 activity in the tumor cells (Figure S13). Further studies of the role of **4** in the p53 and Stat3 pathways are currently underway and will be reported in due course.

Table 3. Antiproliferative Activity of 4 and 1 (IC_{50}, μM)

cell line	IC_{50}	cell line	IC_{50}	cell line	IC_{50}
MCF7 (**4**)	2.0	OVCAR5 (**4**)	1.8	A2780 CisR (**4**)	0.6
MDA468 (**4**)	1.3	OVCAR4 (**4**)	1.4	A2780 (**4**)	0.8
MDA-MB-231 (**4**)	2.2	IGROV-1/Pt (**4**)	1.6	A2780 CisR (**1**)	3.4
T24 (**4**)	1.8	OVCAR3 (**4**)	0.5	A2780 (**1**)	1.8

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00685.

> Experimental procedures and UV, IR, CD, MS, and NMR spectra for **1**−**4**; related original ECD calculation data for epoxyphomalin A, **2**, and **3** (PDF)

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Notes

The authors declare no competing financial interest.

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