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Sphaerialactonam, a γ-lactam–isochromanone from the Hawaiian endophytic fungus *Paraphaeosphaeria* sp. FT462

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ABSTRACT

Reinvestigation of the endophytic fungus *Paraphaeosphaeria* sp. FT462 from the Hawaiian plant *Lycopodiella cernua* (L.) Pic. Serm led to the isolation and identification of a unique γ-lactam-isochromanone (**1**). The structure of **1** was determined by NMR, MS spectroscopic analysis, and quantum chemical calculations of NMR. The compound was tested inactive against A2780 and A2780cisR.

Endophytic fungi are rich sources of new compounds, some of which have different bioactivities.[1–9] Literature search showed that endophytic fungi in Hawaii are underexplored. Therefore, we decided to establish a natural product library, with focus on endophytic fungi in Hawaii. So far, we have collected more than 2500 fungal strains, which were isolated from plants mainly at Oahu, Maui, Kauai, and the Big Island. Our previous study of the endophytic fungus *Paraphaeosphaeria* sp. FT462, a strain isolated from a Hawaiian indigenous plant, *Lycopodiella cernua* (L.) Pic. Serm, yielded two types of uncommon compounds.[6,7] The structures of both lycopodiellactone (an isochromanone)[6] and paraphaeosphaeride A (a pyranone-lactam)[7] were unique, and phaeosphaeride A showed STAT3 inhibition.[7,9] LC/HRMS analysis of a crude sample from FT462 showed that there are some isochromanone and pyranone-lactam analogs in *Paraphaeosphaeria* sp. FT462. Hence, we decided to reinvestigate the strain FT462.

The strain FT462 was cultured in liquid medium under static condition, and the cultured broth was first separated with HP20 as described in our previous publication.[7] Compound **1**[10] was obtained after further purification of a fraction from HP20 with HPLC. Here, we describe the isolation, structure elucidation, proposed biogenetic pathway, and bioactivity of compound **1** (Fig. 1).

Compound **1**[10] was obtained as a colorless powder. Its molecular formula was determined to be $C_{17}H_{17}NO_7$ based on the $[M+H]^+$ ion at *m*/*z* 348.1085 (calcd 348.1083) in the HRESIMS spectrum, which was in good accordance with the ^1H and ^{13}C NMR spectroscopic data (Table 1). The IR spectrum showed the existence of hydroxyl group (3350 cm^{-1}) and carbonyl groups (1613 cm^{-1}). The ^1H NMR spectrum demonstrated the presence of one aromatic proton signal at δ_H 6.32 (1H, s); two olefinic proton signals at δ_H 4.72 (1H, brs) and δ_H 4.62 (1H, brs); two methine signals at δ_H 4.52 (1H, q, J = 6.0 Hz) and δ_H 4.18 (1H, br d, J = 10.0 Hz); three methylene groups at δ_H 4.30 & 4.74 (2H, d, J = 18.0 Hz), δ_H 2.04 & 2.22 (2H, m) and δ_H 2.26 & 2.46 (2H, m); and one methyl signal at δ_H 1.41 (1H, d, J = 6.0 Hz), which were evidenced by the HSQC spectrum. The ^{13}C NMR, HSQC and HMBC spectra displayed eighteen peaks, indicating the existence of three carbonyl carbons at δ_C 167.0, 169.0, and δ_C 176.6; eight aromatic or olefinic carbons at δ_C 164.7, δ_C 163.6, δ_C 157.4, δ_C 146.1, δ_C 111.9, δ_C 100.5 (CH), δ_C 98.1, and δ_C 94.4 (CH_2); two methine, three methylene and one methyl carbons at δ_C 61.4 (CH), δ_C 35.2 (CH_2), δ_C 34.0 (CH), δ_C 29.3 (CH_2), δ_C 23.3 (CH_2), and δ_C 22.3 (CH_3), respectively.

The COSY (Fig. 2) and TOCSY spectra of **1** showed two spin systems in the molecule, δ_H 4.52 – δ_H 1.41 (H-4–H_3-10), and δ_H 4.18 – δ_H 2.04/2.22 – δ_H 2.26/2.46 (H-2′–H_2-3′–H_2-4′). The

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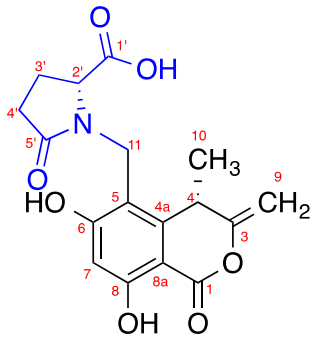

Fig. 1. Structure of compound **1.**

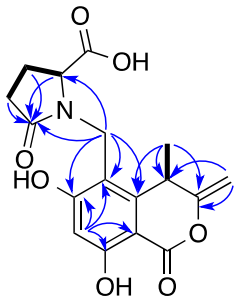

Fig. 2. Key HMBC (arrows) and ^{1}H-^{1}H COSY (bold) correlations of compound **1**.

methylene protons at δ_H 4.30 (Ha-11) and δ_H 4.74 (Hb-11) showed HMBC correlations (Fig. 2) to carbons at δ_C 164.7 (C-6), δ_C 111.9 (C-5) and δ_C 146.1 (C-4a); the aromatic proton at δ_H 6.32 (H-7) correlated to carbons at δ_C 164.7 (C-6), δ_C 163.6 (C-8), δ_C 111.9 (C-5) and δ_C 98.1 (C-8a); the methyl group at δ_H 1.41 (H_2-10) correlated to carbon at δ_C 146.1 (C-4a); both the olefinic methylene at δ_H 4.62/4.72 (H_2-9) and methyl group at δ_H 1.41 (H_2-10) exhibited HMBC correlations to carbons at δ_C 157.4 (C-3) and δ_C 34.0 (C-4), suggesting the presence of an isochromanone moiety like lycopodiellactone (**2**)[6], which was the same as (*S*)-(+)-ascochin (**3**)[11] with the aldehyde group (—CHO) being replaced with a methylene group (—CH_2—). The methylene (δ_H 4.30 and δ_H 4.74) bridge at 11-position demonstrated HMBC correlations to C-2′ (δ_C 61.4) and C-5′ (δ_C 176.6), the later of which was correlated to H-2′ (δ_H 4.18), H-3′ (δ_H 2.04/2.22), and H-4′ (δ_H 2.26/2.46). Based on the molecular formula, the functional group at 2′-position must be a carboxyl group (—COOH), indicating that the methylene (δ_H 4.30 and δ_H 4.74) at 11-position was connected to a pyroglutamic acid moiety. Hence, the planar structure of **1** was determined as shown.

We have previously determined the configuration of lycopodiellactone (**2**)[6] as *S*. From the biogenetic point of view, the configuration at C-4 of compound **1** should be the same as that of compound **2**, though the configuration at C-2′ remained unknown. Given the remote possibility of unveiling this issue by NOESY experiments since no NOESY correlation between H-4 and H-2′ was observed, we decided to determine the relative configuration of **1** using quantum chemical calculations of NMR shifts.[12] We have employed this approach recently to suggest the most likely structure of complex natural products.[13] Among the several strategies that have been developed to determine the most likely stereostructure of organic compounds from theoretical calculations,[12b,14] the DP4 probability is the method of choice when only one set of experimental data is available (as in this case).[12b,14d] Here, we used the DP4+ probability (an improved version of DP4 that takes into account both scaled and unscaled chemical shifts computed at higher levels of theory) to accomplish our goals.[14b] Following the DP4+ general procedure, an exhaustive exploration of the conformational space of the two candidate isomers (**1a** and **1b**, Fig. 3) was carried out with the MMFF force field, and all rotamers were fully optimized at the PCM/B3LYP/6-31G∗ level of

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** in CD_3OD.

	1 (in CD_3OD)			**1** (in CD_3OD)	
No.	δ_C	δ_H	No.	δ_C	δ_H
1	167.0		9	94.4	4.62, brs; 4.72, brs
3	157.4		10	22.3	1.41, d (6.0)
4	34.0	4.52, q (6.0)	11	35.2	4.30, d (18.0); 4.74, d (18.0)
4a	146.1		1′	169.0	
5	111.9		2′	61.4	4.18 br d, (10)
6	164.7		3′	23.3	2.04, m; 2.22, m
7	100.5	6.32, s	4′	29.3	2.26, m; 2.46, m
8	163.6		5′	176.6	
8a	98.1				

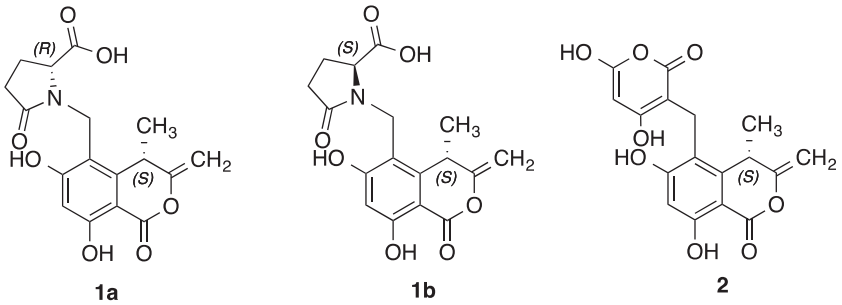

Fig. 3. Structures of **1a** (4*S*2′*R*), **1b** (4*S*2′*S*) and **2.**

Table 2
Calculated ^{1}H and ^{13}C NMR shifts of **1a** and **1b**.

Atom	Exp	Calc[a]	
		1a (4*S*2′*R*)	**1b** (4*S*2′*S*)
H4	4.52	3.56 (4.42)	3.66 (4.60)
H7	6.32	6.63 (6.21)	6.72 (6.23)
H9a	4.62	5.09 (4.89)	4.97 (4.75)
H9b	4.72	5.11 (4.93)	5.08 (4.92)
H10	1.41	1.28 (1.33)	1.28 (1.45)
H11a	4.30	3.76 (4.19)	3.96 (4.01)
H11b	4.74	4.56 (4.67)	4.30 (4.69)
H2′	4.18	4.31 (4.12)	4.34 (4.24)
H3a′	2.04	2.12 (2.07)	2.01 (2.03)
H3b′	2.22	2.37 (2.28)	2.47 (2.19)
H4a′	2.26	2.36 (2.26)	2.58 (2.24)
H4b′	2.46	2.63 (2.43)	2.42 (2.42)
	CMAE	**0.30 (0.09)**	**0.31 (0.08)**
	CMaxErr	**0.96 (0.27)**	**0.86 (0.29)**
C1	167.0	165.4 (166.6)	165.1 (166.7)
C3	157.4	156.8 (158.4)	156.8 (158.3)
C4	34.0	37.1 (36.0)	35.4 (36.1)
C4a	146.1	145.8 (147.7)	145.0 (147.4)
C5	111.9	110.3 (110.1)	110.3 (111.4)
C6	164.7	163.3 (161.8)	164.2 (161.0)
C7	100.5	101.1 (98.5)	99.6 (98.5)
C8	163.6	163.0 (163.4)	162.6 (163.1)
C8a	98.1	96.2 (99.0)	98.1 (99.2)
C9	94.4	94.2 (94.1)	94.1 (94.1)
C10	22.3	21.7 (22.1)	21.1 (22.3)
C11	35.2	36.1 (34.9)	37.9 (33.7)
C1′	169.0	172.8 (174.3)	172.6 (174.7)
C2′	61.4	60.0 (59.7)	62.0 (59.3)
C3′	23.3	23.4 (24.4)	23.7 (24.6)
C4′	29.3	28.7 (29.2)	27.5 (29.5)
C5′	176.6	178.7 (174.7)	179.0 (174.9)
	CMAE	**1.3 (1.4)**	**1.3 (1.5)**
	CMaxErr	**3.8 (5.3)**	**3.6 (5.7)**

[a] The values outside parenthesis were computed from the most stable conformations found at the PCM/B3LYP/6-31G* level. The values in parenthesis were computed after neglecting all conformations with a double H-bonding between the OH groups at C6 and C8 with the carbonyl groups at C5′ and C1, respectively.

theory using methanol as solvent. These geometries were used for further calculations of the NMR shifts with the GIAO method,[15] implemented in Gaussian 09,[16] at the PCM/mPW1PW91/6-31+G** level of theory (Table 2).[14b] Finally, with this data in hand we finally calculated the DP4+ probability and found that isomer **1a** (with a 4*S*2′*R* configuration) was the most likely candidate (99.9%). The ^{13}C NMR data computed for **1a** showed very good agreement with the reported values, with a CMAE (corrected mean average error, defined as $\Sigma_n|\delta_{scaled} - \delta_{exp}|/n$) and CMaxErr (corrected maximum error, defined as $\max|\delta_{scaled} - \delta_{exp}|$) values of 1.3 and 3.8 ppm, respectively. On the other hand, we observed a more modest match when analyzing the ^{1}H NMR shifts, with CMAE values of 0.30 and 0.31 ppm for **1a** and **1b**, respectively. Despite the DP4+ probabilities computed using only ^{13}C (C-DP4+) or ^{1}H (H-DP4+) data pointed toward the same direction (99.4% and 83.5% in favor of **1a**, respectively), the large outliers observed in the predicted ^{1}H NMR values (for example, H-4 and Ha-11) caught our attention. First, we recomputed the shielding tensors at higher levels of theory (PCM/mPW1PW91/6-311++G(2d,p)) and obtained similar results that those collected using at the 6-31+G** level. Moreover, to further validate the level of theory employed in this work, we computed the NMR shifts of a related natural product (ascochin, **3**) and obtained good reproducibility both for ^{1}H and ^{13}C NMR data (see SI). Thus, we speculated that the source of the discrepancy was conformational. The most stable (and highly populated after Boltzmann analysis) conformations of **1a** and **1b** located at the PCM/B3LYP/6-31G* level were those bearing a double hydrogen bonding between the OH groups at C-6 and C-8 with the carbonyl groups at C-5′ and C-1, respectively. Despite such description of the system would probably fit in less polar solvents (such as $CDCl_3$), in CD_3OD (the solvent employed in this study) a wide variety of more flexible conformations would be expected as well.[12a] This represents a common source of error in the NMR calculations of polar molecules in protic solvents, and this is the reason why chloroform is a much convenient solvent to model.[12a] Unfortunately, all attempts to acquire the NMR spectra of **1** in $CDCl_3$ were unsuccessful because of its low solubility in that solvent. Nevertheless, to test our hypothesis we computed the NMR shifts after neglecting all the double H-bonded conformations and a much better agreement between experimental and calculated ^{1}H NMR values was noted (CMAE = 0.09 ppm and CMaxErr = 0.27 ppm for **1a**), as indicated in Table 2 (values in parenthesis). In either way, with these new shifts isomer **1a** was again identified as the most likely candidate in good confidence (DP4+ = 99.3%). Therefore, on the basis of the computational work herein reported we suggest that the correct configuration of **1** should be 4*S*2′*R*. Nevertheless, considering the separation and disconnection of the two stereocenters in the target molecule, the other diastereoisomer should not be irrefutably rejected, and further synthetic work might be needed to ultimately solve the structure of sphaerialactonam.

A possible biogenetic pathway for compound **1** is proposed as shown in Fig. 4. The nitrogen in glutamic acid or pyroglutamic acid

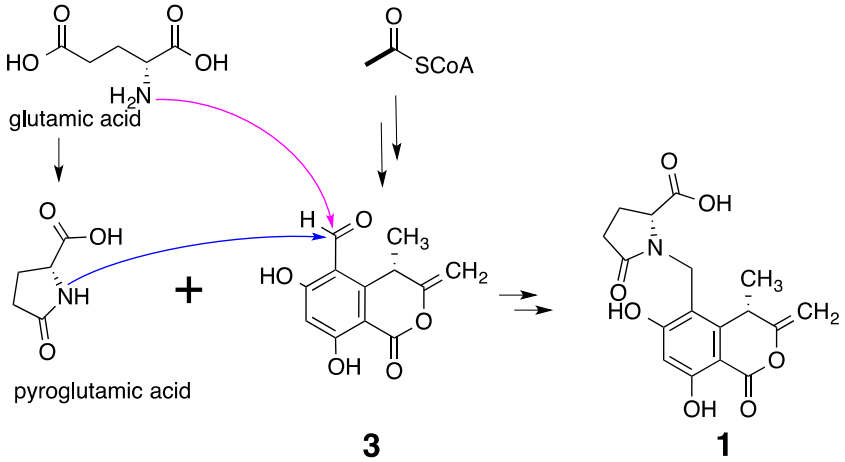

Fig. 4. Hypothetical Biosynthesis for compound **1.**

undergoes nucleophilic addition with the aldehyde carbon of compound **3**, which leads to the formation of the condensation product, **1**.

Like compound **2**, compound **1** is another addition to a rare group of natural 3-methyleneisochromenone derivatives, for exmples, (*S*)-(+)-ascochin (**3**),[11] (*R*)-3,4-dihydro-4,6,8-trihydroxy-4,5-dimethyl-3-methyleneisochromen-1-one,[17] (*R*)-3,4-dihydro-4,8-dihydroxy-5-hydroxylmethyl-6-methoxyl-4-methyl-3-methyleneisochromen-1-one,[18] (*R*)-3,4-dihydro-4,8-dihydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one, halorosellinas A and B,[19] phomopsilactone,[20] and (*R*)-3,4-dihydro-4-hydroxyl-6,8-dimethoxyl-4-methyl-3-methyleneisochromen-1-one.[21] Compound **1** is the first uncommon natural product with both a γ−lactam and methylene isochromenone moiety.

Compound **1** showed no activity (IC_{50} > 20 μg/mL) when tested for its anti-proliferative activity against A2780 (human ovarian cancer cell line) and A2780cisR (cisplatin-resistant human ovarian cancer cell line) by the CyQuant assay.[7]

Conflict of interest

The authors declare no competing financial interest.

Acknowledgment

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.02.052.

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10. a. Isolation and Identification of Fungal Strain.[6]b. Cultivation.[6]c. Isolation of compound **1**: The fermented whole broth (9 L) was filtered through filter paper to separate the supernatant (part i) from the FT462 mycelia. The filter residue, which contained the mycelia, was extracted twice by 80% Acetone/H_2O, then the aqueous acetone solution was concentrated under reduced pressure to afford an aqueous solution (part ii). Subsequently, the combination liquid of the former supernatant solution (part i) and the mycelia extraction (part ii) was passed through Diaion HP-20 eluted with MeOH-H_2O (10%, 50%, 70%, 90%) to afford four fractions (A–D). Fraction B (420 mg) was further fractionated by preparative HPLC (C18 column, 5 μm; 100.0 × 21.2 mm; 10 mL/min; with 0.1% formic acid in mobile phases) eluted with 10–100% MeOH-H_2O to get 30 subfractions (B1-B30). B28 (41.6 mg) was purified by semi-preparative HPLC (Phenomenex luna C18 column, 5 μm; 250.0 × 10.0 mm; 3 mL/min; with 0.1% formic acid in 55% MeOH/H_2O) to obtain compound **1** (1.16 mg, t_R 28.2 min).d. General Experimental Procedures.[6] Sphaerialactonam (**1**): colorless powder; $[\alpha]_D^{25}$ = +101.6 (*c* = 0.2, CH_3OH); UV (MeOH) λ_{max} 221, 272, 307 nm; IR (film) ν_{max} 3350, 2157, 2002, 1977, 1613, 1353 cm^{-1} ^1H NMR (400 MHz, CD_3OD): see Table 1; ^{13}C NMR (100 MHz, CD_3OD): see Table 1; HRESIMS *m/z* 348.1085 [M+H] (calcd for $C_{17}H_{18}NO_7$, 348.1083).
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