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Arthpyrones A–C, Pyridone Alkaloids from a Sponge-Derived Fungus *Arthrinium arundinis* ZSDS1-F3

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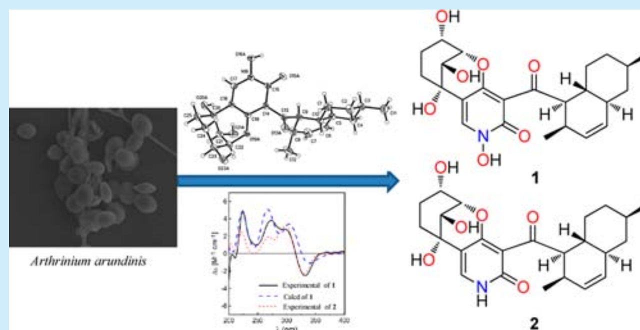
Supporting Information

ABSTRACT: Three new 4-hydroxy-2-pyridone alkaloids, arthpyrones A–C (**1–3**), were isolated from the sponge-derived fungus *Arthrinium arundinis* ZSDS1-F3. Their structures were elucidated on the basis of spectroscopic analysis, CD spectra, quantum chemical calculation, and X-ray single-crystal diffraction analysis. Compounds **1** and **2** possessed a 2-pyridone core featured with a decalin moiety linked via a carboxide bridge bearing a novel oxabicyclo[3.3.1]-nonane ring system rarely discovered in nature. A possible biosynthetic pathway for them was proposed.



With more than 8600 bioactive metabolites described, cultured fungi have been a prolific resource for drug discovery.[1] Terrestrial fungi are known to be rich sources of biologically active compounds for medicinal and agricultural applications.[2] Unfortunately, beginning in the late 1980s, the rate of discovery of new drug candidates from terrestrial microorganisms began to decrease.[3] Due to the frequent rediscovery of the known fungal metabolites from common habitats, fungi from special ecological niches have attracted the attention of natural product researchers.[4] Sponge-derived fungi produce unique and biologically active secondary metabolites.[5]

In order to search for new bioactive compounds, marine sponge samples were collected from the Xisha Islands of China. From a marine sponge sample, *Phakellia fusca* Thiele, the fungal strain ZSDS1-F3, which was identified as *Arthrinium arundinis* (Figure S1, Supporting Information), was isolated and selected for chemical study because its secondary metabolites showed cytotoxic activity against K562 and A549 cell lines at a concentration of 100 μg/mL. Other strains of *Arthrinium* sp. had been reported to show antiviral (HIV) activity and produce a new syncytium formation inhibitor.[6] A series of metabolites contained in the extract of strain ZSDS1-F3 showed UV absorptions similar to those of 4-hydroxy-2-pyridone alkaloids, such as *N*-hydroxyapiosporamide (**4**)[7] and didymellamides A–D[8] by HPLC–UV analysis at 280 and 320 nm. Additionally, 4-hydroxy-2-pyridone alkaloids are known to have cytotoxic, antifungal, antibacterial, and cholesterol esters transfer protein activities as well as induction of neurite outgrowth.[8,9] Chemical investigation resulted in the isolation of three new 4-hydroxy-2-pyridone alkaloids, which we have named arthpyrones A–C (**1–3**, 28.9, 6.4, and 8.0 mg, respectively) (Figure 1). One

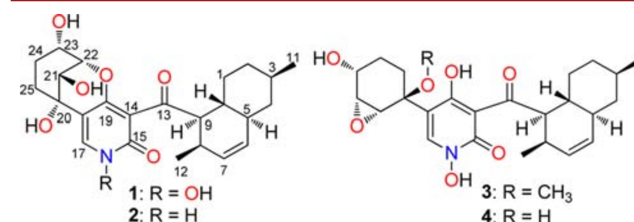


Figure 1. Structures of compounds **1–4**.

known pyridone alkaloid analogue, *N*-hydroxyapiosporamide (**4**) (533.2 mg),[7] was also isolated. Compounds **1–4** were tested for their cytotoxic and antiacetylcholinesterase activities (Tables S1 and S2, Supporting Information).

Arthpyrone A (**1**) gave an HRESIMS peak at m/z 446.2169, corresponding to the molecular formula $C_{24}H_{31}NO_7$. Its IR spectrum exhibited absorptions at 3321 cm^{-1} (hydroxy), 1682 and 1639 cm^{-1} (carbonyl). The ^{13}C NMR (Table 1), DEPT, and HMQC spectra revealed the presence of 24 carbons, namely, two methyls, five methylenes, eight methines, one

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Table 1. ^{1}H and ^{13}C NMR Data for 1−3 (500, 125 MHz, CD_3OD, TMS, δ, ppm)

position	1 δ_C	1 δ_H (J, Hz)	2 δ_C	2 δ_H (J, Hz)	3 δ_C	3 δ_H (J, Hz)
1	30.2, CH_2	1.96, d, (11.8)	30.2, CH_2	2.00, dd (12.9, 2.7)	31.0, CH_2	1.90, m
		1.12, m		1.16, qd (12.0, 2.8)		0.88, m
2	36.7, CH_2	1.70, m	36.5, CH_2	1.79, dd (13.4, 4.0)	36.6, CH_2	1.73, m
		0.97, t (12.3)		1.00, qd (13.1, 3.6)		1.02, m
3	34.3, CH	1.47, m	34.4, CH	1.49, m	34.4, CH	1.48, m
4	43.1, CH_2	1.67, m; 0.73, q (12.1)	43.2, CH_2	1.70, m; 0.75, q (12.0)	43.1, CH_2	1.74, m; 0.76, m
5	43.4, CH	1.81, m	43.4, CH	1.86, m	43.2, CH	1.80, m
6	131.7, CH	5.30, d (9.6)	131.4, CH	5.33, d (9.8)	131.7, CH	5.38, d (9.3)
7	132.7, CH	5.49, brs	132.5, CH	5.49, ddd (9.7, 4.6, 2.7)	132.7, CH	5.56, brs
8	33.5, CH	2.52, brs	33.6, CH	2.55, m	32.4, CH	2.85, m
9	56.7, CH	3.67, m	56.7, CH	3.70, brs	54.5, CH	4.49, m
10	38.1, CH	1.80, m	38.1, CH	1.47, m	37.6, CH	1.55, m
11	23.0, CH_3	0.88, d (6.3)	23.0, CH_3	0.91, d (6.4)	22.9, CH_3	0.92, d (6.5)
12	18.1, CH_3	0.88, d (6.3)	18.1, CH_3	0.92, d (6.4)	18.4, CH_3	0.80, d (3.6)
13	207.2, C		208.4, C		211.9, C	
14	112.7, C		112.9, C		108.3, C	
15	159.1, C		164.7, C		160.6, C	
17	135.2, CH	7.84, brs	136.4, CH	7.53, brs	142.5, CH	8.38, brs
18	113.0, C		114.4, C		108.9, C	
19	162.0, C		164.9, C		176.4, C	
20	70.5, C		70.6, C		76.1, C	
21	69.6, CH	3.70, brs	69.6, CH	3.71, dd (11.2, 5.5)	60.4, CH	3.85, d (2.9)
22	85.4, CH	4.53, brs	85.5, CH	4.53, brs	58.1, CH	3.38, t (2.7)
23	71.5, CH	3.79, m	71.7, CH	3.79, ddd (12.1, 5.3, 1.7)	68.0, CH	4.08, m
24	27.8, CH_2	1.82, m; 1.28, m	27.8, CH_2	1.83, m; 1.31, m	25.3, CH_2	1.76, m; 1.29, m
25	37.4, CH_2	1.71, m; 1.44, m	37.5, CH_2	1.74, m; 1.72, m	28.6, CH_2	2.38, m; 1.57, m
20-OCH_3					50.8, CH_3	3.10, s

oxygenated quaternary carbon, three olefinic methines, two carbonyls, and three other sp^2 quaternary carbons, which accounted for the 10 degrees of unsaturation. In the ^{1}H NMR spectrum, the most salient signals were for two methyl groups at δ_H 0.88 (d, H-11) and 0.88 (d, H-12), two olefinic protons at δ_H 5.30 (d, H-6) and 5.49 (brs, H-7), a singlet at δ_H 7.84 (brs, H-17), and three oxygen-bearing methine at δ_H 4.53 (brs, H-22), 3.79 (m, H-23), and 3.70 (brs, H-21). Comparison of UV−vis and ^{1}H and ^{13}C NMR data with those of *N*-hydroxyapiosporamide (4)[7] revealed a high degree of similarity, especially sharing the same molecular formula. Detailed comparison of NMR data of these two compounds suggested that they had the same decalin ring. The main differences between 1 and 4 occurred in the α-1,4-dihydroxypyridone and cyclohexane moieties. The COSY correlations from H-21 to H-22, H-22 to H-23, H-23 to H_2-24, and H_2-24 to H_2-25 and the HMBC correlations from H-21, H_2-24, and H_2-25 to C-20 suggested the presence of a cyclohexane moiety. However, the chemical shifts C-19, C-21, and C-22 of compound 1 (δ_C 162.0, 69.6, and 85.4, respectively) were observed, which did not match with data for 4-hydroxypyridone and cyclohexane moieties of *N*-hydroxyapiosporamide (4) (δ_C 175.5, 60.4, and 57.6, respectively), indicating the absence of the epoxide group. These two atoms (C-19 and C-22) were found to be directly connected via ether linkage, which was further supported by the key HMBC correlation from H-22 to C-19 (Figure 2), consistent with the degrees of unsaturation and molecular formula. The NOESY correlations of H-5/H-9, H-10/H_3-12 indicated *trans*-diaxial-like relationships of H-5/H-10, H-9/H-10, and H-9/H_3-12. Additional NOESY correlations of H-1/H-3 and H-5 located at H-3, H-5, H-8, and H-9 on the same face,

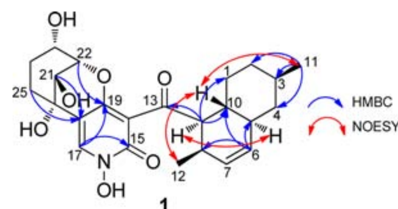

Figure 2. Key HMBC and NOESY correlations of compound 1.

which positioned H-10, H_3-11, and H_3-12 on the opposite face (Figure 2). In order to verify the proposed structure, compound 1 was subjected to single-crystal X-ray diffraction analysis (Figure 3). Bearing on seven oxygen atoms in the molecule, the final refinement on the Cu Kα data resulted in a Flack parameter,[10] 0.0(5), which determined the absolute stereochemistry of 1 to be 3*R*,5*S*,8*R*,9*R*,10*R*,20*S*,21*S*,22*S*,23*S*.

The molecular formula of arthpyrone B (2) was determined to be $C_{24}H_{31}NO_6$ based on the HRESIMS peak at *m/z* 428.2069 $[M - H]^-$, with one oxygen atom less than 1. The [1]H

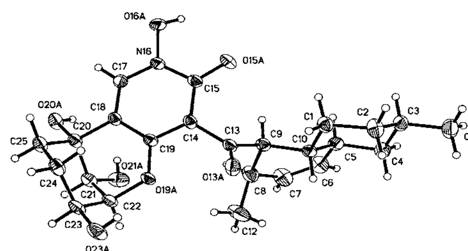

Figure 3. ORTEP drawing of compound 1 (Cu Kα).

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and ^{13}C NMR data of **2** were almost the same as those of arthpyrone A (**1**). However, the α-1,4-dihydroxypyridone moiety of compound **2** did not match with data for the 4-hydroxypyridone moiety of arthpyrone A (**1**). The ^{1}H NMR chemical shifts for H-17 differed significantly (**2** δ_H 7.53; **1** δ_H 7.84), supporting the absence of an *N*-hydroxy group in **2**. The key COSY and HMBC correlations for the decalin ring and cyclohexane moiety were in good agreement with the data for **1**. The similarity of CD curve [CD (*c* 0.02, MeOH) ($\Delta\varepsilon_{max}$) 225 (+5.47), 274 (+4.33), 295 (+3.21), 331 (−2.80)] of **2** with those of **1** [CD (*c* 0.02, MeOH) ($\Delta\varepsilon_{max}$) 223 (+2.84), 271 (+2.29), 299 (+3.78), 331 (−2.64)] indicated the same absolute configuration between **2** and **1** (Figure 4).

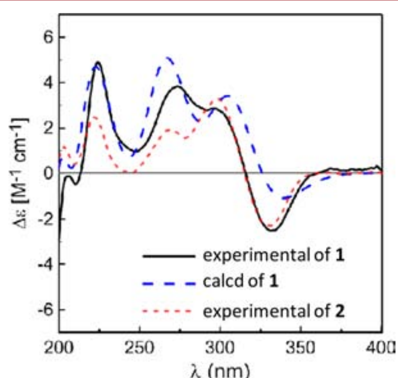
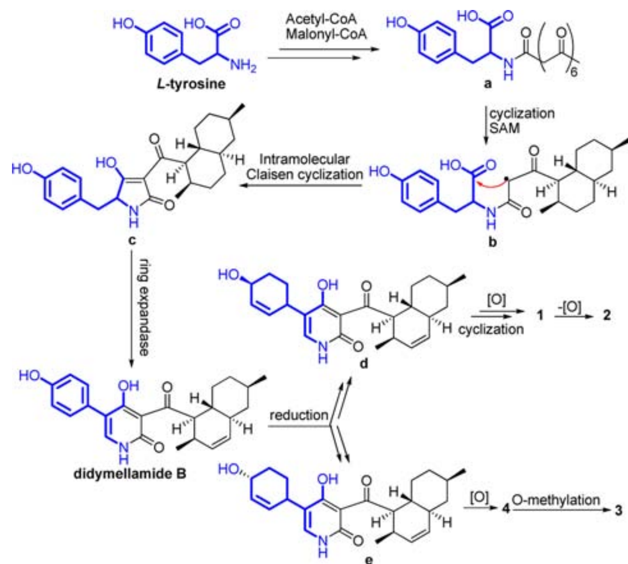


Figure 4. Comparison between calculated (CAM-B3LYP/SVP) and experimental ECD spectra of **1** and **2** in MeOH.

Arthpyrone C (**3**) was isolated as a yellow amorphous solid. Its molecular formula was determined as $C_{25}H_{33}NO_7$ on the basis of HRESIMS, with 10 degrees of unsaturation. Detailed analysis of the 1D- and 2D-NMR spectral data revealed that **3** were very similar to those of **4**,[7] indicating that they shared the same skeleton. The main difference in the ^{1}H NMR spectrum was the presence of a methoxy group at δ_H 3.10 for 20-OCH_3 in **3**, and in the ^{13}C NMR spectrum, a methoxy group at δ_C 50.8 for 20-OCH_3 was observed in **3** instead of a hydroxy group located at C-20 in **4**. This deduction was further supported by the HMBC correlation of 20-OCH_3 with C-20. The spectral data of (+)-apiosporamide ($[\alpha]^{24}_D$ +108), which has been established via synthesis,[11] proved to be identical to that reported for the naturally occurring apiosporamide ($[\alpha]_D$ −97.4), but the optical rotation was opposite.[7,11] These results implied that they are enantiomers, and then the naturally occurring apiosporamide was designated as 3*R*,5*S*,8*R*,9*R*,10*R*,20*S*,21*R*,22*R*,23*R*. Detailed analysis of the NMR spectral data revealed that the spectrum of *N*-hydroxyapiosporamide (**4**) ($[\alpha]^{25}_D$ −56.3) was very similar to that of naturally occurring apiosporamide,[7,11] and the two should have the same the absolute configurations. In addition, the CD curve [CD (*c* 0.02, MeOH) ($\Delta\varepsilon_{max}$) 278 (+2.57), 314 (+2.64) and specific rotation ($[\alpha]^{25}_D$ −68.0)] of **3** compared with those of **4** [CD (*c* 0.02, MeOH) ($\Delta\varepsilon_{max}$) 215 (−2.14), 272 (+3.43), 314 (+4.86) and specific rotation ($[\alpha]^{25}_D$ −56.3)] were consistent with those of the calculated ECD and experimental CD spectra of **3** and **4** in MeOH (Figure S7, Supporting Information). Therefore, compound **3** was determined as 3*R*,5*S*,8*R*,9*R*,10*R*,20*S*,21*R*,22*R*,23*R*, consistent with the absolute configurations of *N*-hydroxyapiosporamide (**4**).

The four 4-hydroxy-2-pyridone alkaloids **1**−**4** are probably biosynthesized via PKS and amino acid hybrid biogenetic pathways (Scheme 1). In the putative biogenesis of **1**−**4**, the

Scheme 1. Plausible Biosynthetic Pathway of 1−4



linear polyketide precursor initially condenses with an activated L-tyrosine to form intermediate **a** that further produced intermediate **b** with a decalin ring after cyclization. The intermediate **b** is released as a tetramic acid core throuth a catalyzed intramolecular Claisen cyclization to form **c** and then a series of subsequent transformations, including ring enlargement, reduction, hydroxylation, cyclization, dehydration, and O-methylation that lead to the production of arthpyrones A−C (**1**−**3**) and *N*-hydroxyapiosporamide (**4**).[12]

Prior to this study, only nine 4-hydroxy-2-pyridone alkaloids with a decalin ring have been described, including lilicicolin H,[9] fischerin,[13] apiosporamide,[7] *N*-hydroxyapiosporamide (**4**),[7] YM-215343,[9] and didymellamides A−D.[8] All of them were isolated from plant-derived or animal-derived fungi, while the absolute configurations for the decalin ring were identical with each other by the key NOESY correlations and X-ray single-crystal diffraction. Furthermore, the absolute configurations of YM-215343 and (+)-apiosporamide were revealed by total synthesis in 2005.[11] Although the absolute configurations for the decalin ring systems are identical, the absolute configurations for the cyclohexanol moieties are reversed between **1**, **2** and **3**, **4**. The cyclohexanol moieties of **1**−**4** are derived from the benzene ring of tyrosine, and there are several paths of reduction or oxidation about benzene ring (Scheme 1), which may be the reason that their configurations of the cyclohexanol moieties are opposite. Compounds **1** and **2** possessed a 2-pyridone core featured with a decalin moiety linked via a carboxide bridge and bearing a novel oxabicyclo[3.3.1]nonane ring system rarely discovered in nature. Consistent with previous reports, the new members of the 4-hydroxy-2-pyridone alkaloids with decalin ring **1** and **3**, as well as the known compound **4**, had significant in vitro cytotoxicities against the K562, A549, Huh-7, H1975, MCF-7, U937, BGC823, HL60, Hela, and MOLT-4 cell lines, with IC_{50} values ranging from 0.24 to 45 μM (see Table S1, Supporting Information). Furthermore, compound **3** displayed significant AchE inhibitory activity (IC_{50} 0.81 μM), whereas compounds **1**

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and **4** revealed modest activities (IC_{50} 47 and 39 μM, respectively) (see Table S2, Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

ITS gene sequence data of ZSDS1-F3, computational details, isolation, purification, bioassay protocols, and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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