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NOTE

Cycloexpansamines A and B: spiroindolinone alkaloids from a marine isolate of *Penicillium* sp. (SF-5292)

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Marine microorganisms are recognized as an important source of structurally diverse bioactive secondary metabolites.[1,2] Particularly, secondary metabolites from marine fungal isolates have attracted significant interest, as many of them provide unique structural features and interesting biological properties.[2–4] In our search for new metabolites from marine-derived fungi, we recently reported the isolation of an anti-inflammatory metabolite named penicillinolide A from extracts obtained from cultures of a marine-derived isolate of *Penicillium* sp. (SF-5292).[5] As part of our continuing efforts to explore the chemistry of this isolate, studies of extracts obtained from larger-scale cultures of the fungus were undertaken. From this investigation, two minor metabolites, cycloexpansamines A (**1**) and B (**2**) were isolated (Figure 1) in addition to previously encountered penicillinolide A.

The fungal strain was cultured on 80 petri-dish plates (90 mm), each containing 20 ml of potato dextrose agar media (0.4% (w/v) potato starch, 2% (w/v) dextrose, 3% (w/v) NaCl, 1.5% (w/v) agar). Plates were individually inoculated with 2-ml seed cultures of the fungal strain. Plate cultures were incubated at 25 °C for a period of 14 days. Extraction of the agar media with EtOAc (2 × 500 ml) provided an organic phase, which was then concentrated *in vacuo* to yield 1.9 g of an extract. The EtOAc extract was subjected to C_{18} flash column chromatography (5 × 40 cm), eluting with a stepwise gradient of 20, 40, 60, 80 and 100% (v/v) MeOH in H_2O (500 ml each). The fractions eluted at 80% MeOH (418 mg) were purified by semi-preparative reversed-phase HPLC eluting with a gradient from 40 to 100% MeOH in H_2O (0.1% formic acid) over 56 min to yield compound **1** (10.2 mg, $t_R = 15.9$ min). The fraction collected between 17 and 18 min in the above HPLC step was then subjected to further HPLC, eluting with a gradient from 40 to 65% MeOH in H_2O (0.1% formic acid) over 40 min to yield compound **2** (1.4 mg, $t_R = 27.6$ min).

Cycloexpansamine A (**1**) gave an $[M+H]^+$ ion at *m/z* 450.2380 in the HRESI mass spectrum, indicating a molecular formula of $C_{26}H_{31}N_3O_4$, which was fully supported by the ^1H and ^{13}C NMR data (Table 1). Analysis of its ^{13}C NMR and DEPT spectra revealed the presence of four methyl groups, two sp^2 methines, one sp^3 methine and seven sp^3 methylene units (including one bound to a hetero-atom), as well as three carbonyl and four non-protonated aromatic/olefinic carbons. Because only six out of the 13 unsaturations were accounted for by these units, cycloexpansamine A (**1**) was required to contain seven rings. The structure of **1** could be fully defined by comprehensive analysis of 1D and 2D NMR data, including ^1H, ^{13}C NMR, COSY, HSQC and HMBC experiments (Table 1). A literature search indicated that the planar structure of **1** was proposed for a metabolite of another unidentified *Penicillium* sp. in a recent US patent.[6] However, ^1H and ^{13}C NMR position assignments were not provided, nor were HRMS data and stereochemical assignments. Therefore, our attention turned into the comprehensive structure determination of **1**, including absolute configuration.

The relative configuration of compound **1** was determined by analysis of NOESY data (Figure 2). Since **1** consists of separated fused ring systems connected at a spiro-carbon atom, the two ring systems are virtually perpendicular to one another. NOESY correlations of H-10 with H_3-28 and H-23β, and of H-23β with *N*H-25 indicated that one of the methyl groups at C-13, the C1 − C11 bond and the amide group (C-24) are β-oriented. This was also supported by the observation of NOESY correlations of H_3-28 with H-23β. On the other hand, NOESY correlations of H_3-29 with H-14; of H-14 with H-21α and H-23α; and of H-21α with H-23α suggested α-orientations for H-14, H-21α, H-23α and H_3-29. Therefore, the relative configuration of **1** was suggested as shown.

The absolute configuration of **1** was proposed by analysis of its electronic circular dichroism (ECD) spectrum and by comparison to its calculated spectrum (Supplementary Information). The Cotton effect at 250–350 nm is considered to be related to the configuration at the spirogenic center in ECD spectra of spiro-oxindole alkaloids.[7–9] The ECD spectrum of **1** (Supplementary Figure S1) showed a positive Cotton effect in the spiro-oxindole absorbance region around 270 nm, which suggested the 1*S* configuration for **1**, and this assignment is in agreement with the configurations previously established for related

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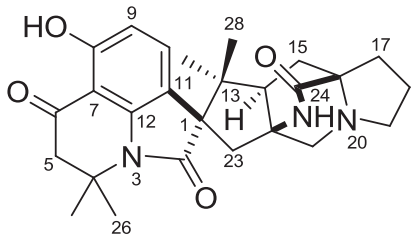

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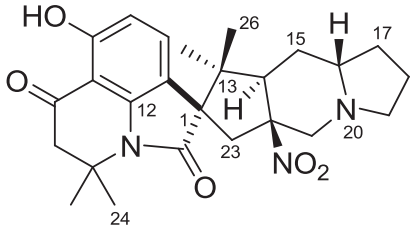

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Figure 1 Structures of cycloexpansamines A (**1**) and B (**2**).

Table 1 NMR spectroscopic data for cycloexpansamine A (1) in pyridine-d_5

No.	δ_C[a]	δ_H, *mult. (J in Hz)*[b]	*Key NOESY*[c]	*HMBC (H→C#)*
1	64.9	—	—	
2	183.0	—	—	—
4	57.2	—	—	—
5	51.6	2.67, d (16.5)	—	
		2.99, d (16.5)	—	4, 6, 7, 26, 27
6	197.5	—	—	—
7	104.9	—	—	—
8	160.0	—	—	—
9	108.8	6.60, d (8.4)	—	6, 7, 8, 11
10	134.3	7.26, d (8.4)	H-23β, H-28,	1, 8, 12
11	119.2	—	—	—
12	147.9	—	—	—
13	47.8	—	—	—
14	49.3	3.39, t (8.4)	H-15α, H-21α, H-29,	13, 15, 21, 22, 28, 29
15	32.7	(α) 1.90, m	H-14	14, 16, 17, 22, 24
		(β) 1.78, m	H-28	—
16	67.3	—	—	—
17	27.8	3.01, m	—	16, 24
		1.51, m	—	15, 16, 24
18	24.9	1.86–1.99, m	—	17, 19
19	54.0	3.06, m	—	16
		2.28, dd (17.2, 8.4)	—	18, 21
21	64.0	(α) 3.75, d (8.4)	H-23α	14, 15, 16, 22, 23
		(β) 2.49, d (8.4)	—	14, 19, 22
22	63.5	—	—	—
23	40.4	(β) 2.47, d (15.0)	H-10, H-25, H-28	2, 11
		(α) 2.38, d (15.0)	H-21α	1, 2, 13, 21
24	174.0	—	—	—
25	—	9.06, s	H-23β	14, 16, 22, 23
26	23.9	1.43, s	—	4, 5, 27
27	26.8	1.77, s	—	4, 5, 26
28	21.3	1.12, s	H-10, H-15β, H-23β	1, 13, 14, 29
29	23.3	0.84, s	H-14	1, 13, 14, 28

[a]Recorded at 100 MHz.
[b]Recorded at 400 MHz.
[c]Recorded at 600 MHz

compounds containing spiro-oxindole units, such as synthetic *ent*-(+)-paraherquamide B[10] and (+)-versicolamide B.[7] In an effort to provide independent support for this assignment, the ECD spectrum of an energy-minimized conformer of **1** (generated using Spartan '10, Wavefunction, Inc., CA, USA) was simulated using time-dependent density functional theory (TDDFT) calculations, and compared with the experimental data for **1**. The experimental ECD spectrum of **1** matched closely with the calculated ECD spectrum. Therefore, on the basis of both empirical considerations and TDDFT calculations, the absolute configuration of **1** was assigned as shown.

Cycloexpansamine B (**2**) was assigned the molecular formula $C_{25}H_{31}N_3O_5$ (11 unsaturations) on the basis of high-resolution electrospray ionisation mass spectrometry (HRESIMS) and NMR data (Table 2). Analysis of its ^{13}C NMR and DEPT spectra revealed the presence of four methyl groups, two sp^2 methines, two sp^3 methines and seven sp^3 methylene units, as well as two carbonyl and four non-protonated aromatic or olefinic sp^2 carbons. Analysis of the ^1H and ^{13}C NMR data suggested that the overall structure of **2** is similar to that of **1**. The main difference was evident in the absence of one carbonyl signal in the ^{13}C NMR spectrum, and changes in the chemical shifts corresponding to the C-15, C-16 (sp^3 methine instead of sp^3 quaternary carbon), and C-22 positions of **1**. These observations suggested that **2** differs from **1** by the absence of the bridged amide ring system and associated structural modification around this functionality. The planar structure of **2** was finally inferred by considering this information together with a comprehensive analysis of 2D NMR data as well as close comparisons of the data with those reported for related metabolites, cyclopiamines[11,12] and citrinalins.[13]

The relative configuration of **2** was determined by analysis of the NOESY spectrum (Figure 2) and by analogy to cycloexpansamine A (**1**). As in the case of **1**, NOESY correlations of H_3-26 with H-10 and H-23β indicated that one of the methyl groups at C-13, the C1 − C11 bond and H-23β were β-oriented. On the other hand, NOESY correlations of H-14 with H-17α, H_3-27 and H-21α suggested α-orientations for H-14, H-17α and H-21α. This assignment in turn suggested β-orientation for the nitro group at C-22. Finally, NOESY correlations of H-15β with H-16, and of H-15α with H-14, H-17α and H_3-27 suggested β-orientation of H-16. Therefore, the relative configuration of **2** was proposed as shown. In addition, by analogy to the recently proposed biogenesis of nitro group containing prenylated indole alkaloids from bicyclo[2.2.2]diazaoctane-containing metabolites (discussed below), the absolute configuration of **2** was proposed to be analogous to that of **1**.

Cycloexpansamine A (**1**) is a novel heptacyclic spiroindolinone alkaloid consisting of a 4,5-dihydro-1*H*-pyrrolo[3,2,1–ij]quinoline-2,6-dione ring system and an amide-bridged cyclopenta[*f*]indolizidine ring system connected via a spiro-carbon atom. Compound **1** is structurally related to the family of prenylated indole alkaloids containing the bicyclo[2.2.2]diazaoctane ring system as a core structure.[7] This family of natural products, including the brevianamides, paraherquamides, stephacidin, asperparalines, marcfortines, notoamides, malbrancheamides, avrainvillamide and sclerotiamide, has been reported mainly from various fungi of the genera *Aspergillus* and *Penicillium*,[10] and several biological activities, including

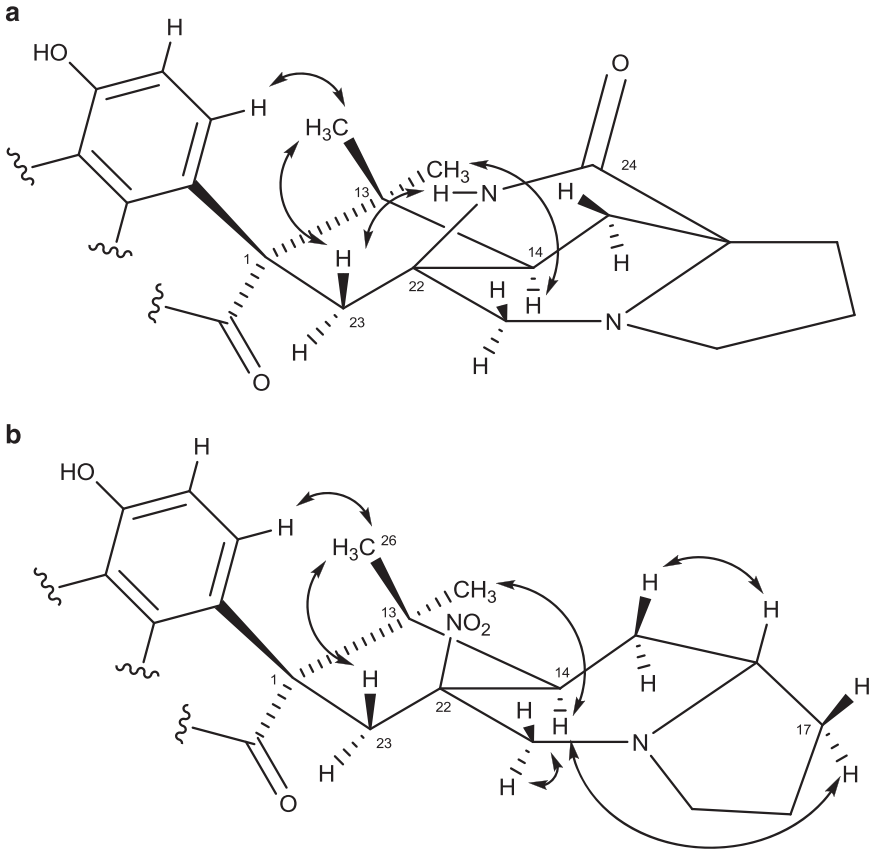

Figure 2 Key NOESY correlations (**a**) for the amide-bridged cyclopenta[*f*]indolizidine portion of cycloexpansamine A (**1**), and (**b**) for the cyclopenta[*f*] indolizidine portion of cycloexpansamine B (**2**).

insecticidal, antitumor, anthelmintic, calmodulin inhibitory and antibacterial properties, are displayed by members of this family.[7] Because of their complex structures and diverse bioactivities, studies of synthetic and biosynthetic pathways leading to these alkaloids has become an area of significant interest.[13–16] In line with this, it is noteworthy that cycloexpansamine A (**1**) represents a rare example of prenylated indole alkaloids with the anti-relative configuration between the C13 – C14 and C16 – N24 bonds in the [2.2.2]diazaoctane core system.[7,17] In addition, cycloexpansamine A (**1**) is the only member of this family that contains a 4,5-dihydro-1*H*-pyrrolo [3,2,1–ij]quinoline-2,6-dione ring system derived from condensation of the indole ring and a prenyl group.

Cycloexpansamine B (**2**) is closely related to cyclopiamines A[11] and B,[11,12] which are the only precedent secondary metabolites possessing a 4,5-dihydro-1*H*-pyrrolo[3, 2, 1–ij]quinoline-2,6-dione ring system. The relative configurations of cyclopiamine B was originally established by single-crystal X-ray crystallography.[11] In the course of the structure elucidation of cyclopiamine B (a diasteromer of cyclopiamine A), the desmethylated analog of cyclopiamine B, which possesses the same planar structure as that of **2**, was prepared by treatment with aluminum chloride in nitrobenzene.[18] Recently, *O*-desmethylcyclopiamine B has been asymmetrically synthesized in the course of total synthesis of cyclopiamine B possessing absolute configuration of 1*R*, 14*R*, 16*R* and 22*S*.[9] The structure of **2** differs from *O*-desmethylcyclopiamine B only in the configurations at stereogenic centers. To confirm the diastereomeric relationship between *O*-desmethylcyclopiamine B and **2**, the NMR data of **2** in $CDCl_3$ were compared with those reported for the asymmetrically synthesized *O*-desmethylcyclopiamine B in the same solvent. Although a comprehensive comparison was hampered by the limited availability of **2,** clear differences in proton shift values for prominent peaks, including the aromatic and methyl proton signals were observed (**2**: aromatic ^{1}H signals at δ_H 7.41 (br d, $J=7.0$, 1H) and 6.57 (br d, $J=7.0$ Hz, 1H); methyl ^{1}H signals at δ_H 1.77 (s, 3H), 1.46 (s, 3H), 0.97 (s, 3H) and 0.80 (s, 3H); *O*-desmethylcyclopiamine B: aromatic ^{1}H signals at δ_H 7.20 (br d, $J=8.3$, 1H) and 6.48 (br d, $J=8.3$ Hz, 1H); methyl ^{1}H signals at δ_H 1.73 (s, 3H), 1.41 (s, 3H), 1.03 (s, 3H) and 0.89 (s, 3H)). These data support the conclusion that **2** is a diastereomer of asymmetrically synthesized *O*-desmethylcyclopiamine B, possessing inverted configurations at C-1, C-14 and C-16 compared to the respective positions in *O*-desmethylcyclopiamine B. It is also noteworthy that encountering fungal metabolites possessing a nitro group is unusual. Cyclopiamines A and B from *Penicillium cyclopium*,[11] cyclopiamin B from *Aspergillus caespitosus*[12] and citrinalins A, B[13] and 17-hydroxycitrinalin B[18] from *P. citrinum* are rare examples of precedent fungal metabolites bearing such functionality. It has been suggested that cyclopiamines and citrinalins biogenetically arise from a bicyclo[2.2.2]diazaoctane precursor *via* hydrolysis of the amide bridge, decarboxylation and amino group oxidation to the nitro group. However, such a precursor was unknown until a recent report of the co-isolation of nitro group containing 17-hydroxycitrinalin B and a bicyclo[2, 2, 2]diazaoctane-containing precursor.[18] Thus, the isolation of cycloexpansamines A and B is be an additional example of co-isolation of biogenetically related metabolites, and this supports the existence of a common bicyclo[2.2.2]diazaoctane-containing biogenetic precursor to nitro group containing prenylated indole alkaloids.

Table 2 NMR spectroscopic data for cycloexpansamine B (2) in pyridine-d_5

No.	δ_C[a]	δ_H, mult. (*J* in Hz) [b]	Key NOESY [c]	HMBC (H→C#)
1	61.8	—	—	
2	182.3	—	—	—
4	57.0	—	—	—
5	51.1	2.66, d (16.5)	—	4, 6, 7, 24
		2.99, d (16.5)	—	4, 6, 24, 25
6	197.0	—	—	—
7	104.7	—	—	—
8	159.7	—	—	—
9	108.9	6.68, d (8.3)	—	7, 8, 11
10	134.1	7.28, d (8.3)	H-23β, H-26	1, 8, 12
11	118.6	—	—	—
12	147.5	—	—	—
13	47.4	—	—	—
14	47.5	3.15, m	H-15α, H-17α, H-21α, H-23α, H-27	15, 21
15	22.4	(β) 2.95, m	H-16, H-26	13, 16, 17, 22
		(α) 1.61, m	H-14, H-17α	13, 16, 22
16	58.8	3.16, dd (9.9, 4.3)	H-15β	13, 15, 21
17	27.5	(α) 1.69, m	H-14, H-15α	18
		(β) 1.63, m	H-15β	18
18	22.6	(β) 1.81, m	H-19β	17
		(α) 1.50, m	H-19α	—
19	54.3	(β) 2.70, m	H-18β, H-21β	16
		(α) 2.65, m	H-18α	18, 21
21	60.5	(β) 3.22, d (11.0)	H-19β	14, 16, 22
		(α) 2.98, d (11.0)	H-14	16, 19
22	95.9	—	—	—
23	43.7	(β) 3.08, d (15.8)	H-26	1, 2, 11, 13, 22
		(α) 2.67, d (15.8)	H-14	1, 2, 13, 22
24	23.7	1.39, s	—	4, 5, 25
25	26.5	1.74, s	—	4, 5, 24
26	22.9	0.94, s	H-10, H-15β, H-23β	1, 13, 27
27	23.4	0.96, s	H-14, H-15α	1, 14, 26

[a]Recorded at 150 MHz.
[b]Recorded at 600 MHz.
[c]Recorded at 600 MHz.

Cycloexpansamine A (**1**) moderately inhibited the activity of protein tyrosine phosphatase 1B,[19,20] a promising and a validated therapeutic target to effectively treat type 2 diabetes mellitus and obesity, showing an IC_{50} value of 27.6 μM, while **2** was inactive against protein tyrosine phosphatase 1B at levels up to 100 μM.

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