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Perinadine A, a Novel Tetracyclic Alkaloid from Marine-Derived Fungus *Penicillium citrinum*

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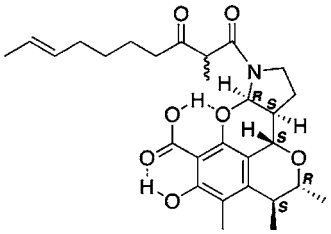
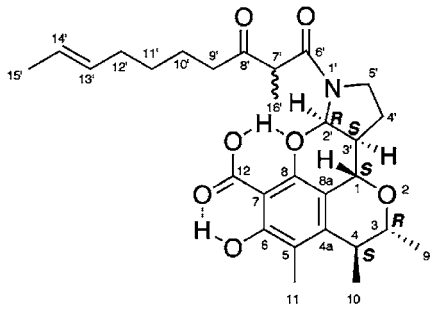
ABSTRACT



perinadine A

A novel tetracyclic alkaloid, perinadine A (1), was isolated from the cultured broth of the fungus *Penicillium citrinum*, which was separated from the gastrointestine of a marine fish, and the structure was elucidated on the basis of spectroscopic data including 2D NMR spectra. Biogenetically, perinadine A (1) may be derived from citrinin (4), a well-known mycotoxin, and a scalusamide A-type pyrrolidine alkaloid.

Marine-derived fungi of the genus *Penicillium* have proven to be a rich source of structurally unique and biologically active secondary metabolites.[1] In our search for new metabolites from marine-derived fungi,[2] three new pyrrolidine alkaloids, scalusamides A (**2**), B, and C, were isolated from the cultured broth of the fungus *Penicillium citrinum*, which was separated from the gastrointestine of an Okinawan marine parrot fish. Recently, a novel tetracyclic alkaloid, perinadine A (**1**), was isolated from the same fungus, and the structure was elucidated on the basis of spectroscopic data including 2D NMR spectra. In this paper, we describe the isolation and structure elucidation of **1**.



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The fungus *Penicillium citrinum* (strain N055) was separated from the gastrointestine of a parrot fish *Scalus ovifrons* collected at Hedo Cape, Okinawa Island, and grown in PYG liquid medium containing seawater for 10 days at 25 °C. The supernatant of the culture broth (12 L) was extracted

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Table 1. ^{1}H and ^{13}C NMR Data of Perinadine A (**1**) in $CDCl_3$

position	δ_C			δ_H	
1	70.14	70.06[a]	CH	4.24 (d, 4.8)	
3	77.68	77.58	CH	3.75 (m)	
4	37.18	37.15	CH	2.82 (dq, 7.2, 7.2)	
4a	144.57		C		
5	120.11	120.15	C		
6	160.98	160.99	C		
6-OH				12.241 (s)	12.235[a] (s)
7	99.96		C		
8	147.26		C		
8a	114.26		C		
9	21.29	21.31	CH_3	1.34[b] (d, 6.7)	1.33[b] (d, 6.7)
10	18.80		CH_3	1.22[b] (d, 7.2)	
11	11.55	11.50	CH_3	2.17[b] (s)	
12	170.78	170.75	C		
12-OH				11.52 (brs)	
2′	86.75	86.87	CH	5.65 (d, 5.4)	5.63 (d, 5.4)
3′	45.92		CH	2.62 (m)	
4′	28.92	28.98	CH_2	2.46 (m)	
				2.26 (m)	
5′	45.44	45.56	CH_2	3.77 (m)	
				3.61 (m)	
6′	170.97	170.93	C		
7′	53.74	53.89	CH	3.61 (m)	
8′	206.52	206.75	C		
9′	38.82	39.29	CH_2	2.58 (m)	2.54[c] (m)
				2.49 (m)	
10′	23.00	22.94	CH_2	1.56[c] (m)	
11′	28.78	28.92	CH_2	1.32[c] (m)	
12′	32.27	32.25	CH_2	1.95[c] (m)	
13′	130.81	130.75	CH	5.33 (m)	
14′	125.16	125.28	CH	5.38 (m)	
15′	17.91		CH_3	1.62[b] (d, 4.8)	1.59[b] (d, 4.8)
16′	13.25	13.44	CH_3	1.44[b] (d, 6.7)	1.47[b] (d, 6.7)

[a] These columns were due to minor signals. [b] 3H. [c] 2H.

with EtOAc, and the EtOAc-soluble portions were subjected to silica gel and amino silica gel column chromatographies followed by C_{18} HPLC ($CH_3CN/H_2O/CF_3CO_2H$) to afford perinadine A (**1**, 5.1 mg). From the other fractions of the EtOAc-soluble portions, scalusamides A[3] (**2**, 10.6 mg), B (1.0 mg), and C (0.9 mg) and a known pyrrolo[2,1-*b*]oxazine compound **3**[4] and its dihydro congener[5] were isolated.

Perinadine A[6] (**1**) was obtained as an optically active colorless solid {$[\alpha]^{22}_D$ −33° (*c* 1.0, $CHCl_3$)}. The molecular formula of **1** was revealed to be $C_{28}H_{37}NO_7$ by negative-mode HRESIMS [*m*/*z* 498.2510, $(M - H)^+$, −0.7 mmu]. The IR spectrum suggested the presence of OH/NH (3405 cm^{-1}), carboxylic acid (3300−2700 cm^{-1}), and carbonyl group(s) (1719 and 1650 cm^{-1}). The ^{13}C NMR (Table 1) spectrum of **1** disclosed two sets of 28 carbon signals due to a ketone (δ_C 206.52 and 206.75), two carbonyl (δ_C 170.97 and 170.93; δ_C 170.78 and 170.75), six sp^2 quaternary carbons (δ_C 160.98 and 160.99; δ_C 147.26; δ_C 144.57; 120.11 and 120.15; both δ_C 114.26; both δ_C 99.96), two sp^2 methines (δ_C 130.81 and 130.75; δ_C 125.16 and 125.28), six sp^3 methines (δ_C 86.75 and 86.87; δ_C 77.68 and 77.58; δ_C 70.14 and 70.06; δ_C 45.44 and 45.56; both δ_C 45.92; δ_C 37.18 and 37.15), the former three of which seemed to be adjacent to a heteroatom, six sp^3 methylenes (δ_C 45.44 and 45.56; δ_C 38.82 and 39.29; δ_C 32.27 and 32.25; δ_C 28.92 and 28.98, δ_C 28.78 and 28.92; δ_C 23.00 and 22.94), and five methyls (δ_C 21.29 and 21.31; both δ_C 18.80; δ_C 17.91; 12.78 and 13.44; δ_C 11.55 and 11.50). The chemical shifts for each set of carbon signals of perinadine A (**1**) were close to each other, probably due to a mixture of epimers at a chiral center (C-7′) like scalusamides.[3] In the deuterium-induced shift experiment of the ^{13}C NMR in $CDCl_3$ by addition of D_2O, the relatively large shifts were observed for a set of carbons resonated at δ_C 170.78 ($\Delta\delta$ 0.19) and 170.75 ($\Delta\delta$ 0.18) and δ_C 160.98 ($\Delta\delta$ 0.23) and 160.99 ($\Delta\delta$ 0.22), thus suggesting the presence of a carboxylic acid and a phenol group. On the other hand, for the relatively low-field sp^2 quaternary carbon at δ_C 147.26 was shown a small induced-shift, thus indicating that this carbon was involved in an ether linkage.

The ^{1}H NMR (Table 1) spectrum included two D_2O-exchangeable protons (δ_H 12.241 and 12.235; δ_H 11.52), in which the lowest-field signal was observed as a sharp singlet, indicating the presence of intramolecular hydrogen bonding. Proton and carbon signals for **1** were assigned by detailed analyses of the HMQC spectrum of **1**.

The gross structure of perinadine A (**1**) was elucidated by spectroscopic data including 2D NMR data such as ^{1}H−^{1}H COSY, NOESY, and HMBC spectra. Four proton networks from H-1 to H-2′ and H_2-5′, from H-3 to H-4, H_3-9, and H_3-10, from H-7′ to H_3-16′, and from H_2-9 to H_3-15 were suggested by analysis of the ^{1}H−^{1}H COSY spectrum (Figure 1). The presence of an *E*-double bond at C-13−C-14 was deduced from the chemical shift of the allylic carbon (C-15, δ_C 17.68).[7] Correlations for H-2′, H_2-5′, and H-7′ to an amide

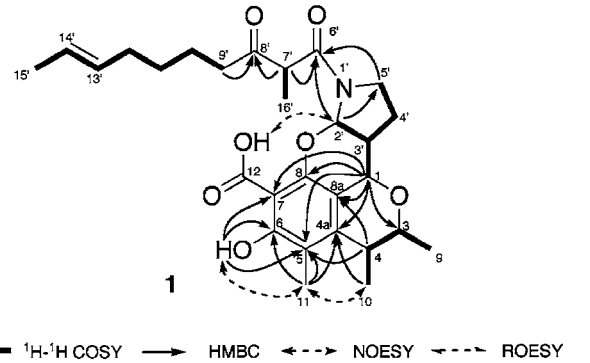

Figure 1. Selected 2D NMR correlations for perinadine A (**1**).

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(6) **Perinadine A (1):** colorless amorphous solid; $[\alpha]^{22}_D$ −33° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3405, 3300−2700 (br), 1719, and 1650 cm^{-1}; UV (MeOH) λ_{max} 315 (ϵ 1700), 252 (3600), and 215 nm (16 000); CD (MeOH) λ_{ext} 313 ($\Delta\epsilon$ −0.5), 257 (+1.3), 224 (−0.6), and 214 nm (+0.6); ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 498 $(M - H)^-$; HRESIMS (*m*/*z* 498.2510 [$(M - H)^-$, calcd for $C_{28}H_{36}NO_7$, 498.2517].

carbonyl carbon (C-6′, δ_C 170.97 and 170.93) suggested that C-7′ was attached to C-2′ and C-5′ through a secondary amide carbonyl group. The existence of a ketone carbonyl (δ_C 206.52 and 206.75) at C-8′ was implied by HMBC correlations for H-7′ and H_2-9′ to C-8′. The relatively lower field chemical shifts for H-2′ (δ_H 5.65 and 5.63) and C-2′ (δ_C 86.75 and 86.87) indicated that another heteroatom in addition to N-1′ was attached to C-2′. The presence of a pyrrolidine ring (N-1′-C-5′) was deduced from the HMBC correlation for H-2′ to C-5′.

The existence of a fully-substituted 2,3*H*-benzopyran skeleton was assigned by HMBC and NOESY correlations as follows. HMBC correlations for H-1 (δ_H 4.24)/C-3 (δ_C 77.68 and 77.58), H-1/C-4a (δ_C 144.57), H-1/C-8a (δ_C 114.26), H-4 (δ_H 2.82)/C-8a, and H_3-10 (δ_H 1.22)/C-4a indicated the presence of 3,4-dimethyl-2,3*H*-pyran ring (C-1-C-4a and C-8a) connected to the pyrolidine ring moiety (N-1′-C-5′) at C-1. From the lower field singlet methyl signal at δ_H 2.17 (H_3-11), HMBC correlations to C-4a, C-5 (δ_C 120.11 and 120.15), and C-6 (δ_C 160.98 and 160.99) were observed, thus indicating that C-11 was attached to C-5. The methyl proton signal of C-11 showed NOESY correlations to H_3-10 and a phenol proton (δ_H 12.241 and 12.235), the latter of which was correlated to C-5, C-6, and C-7 (δ_H 99.96) in the HMBC spectrum. This suggested that the phenolic hydroxyl group was connected to C-6. The methine proton of C-1 gave a three-bond correlation for C-8 (δ_C 147.26), which was associated with an ether linkage, and four-bond HMBC correlations to C-5 and C-7. A residual carboxyl carbon (C-12; δ_C 170.78 and 170.75) was suggested to be attached to C-7 because of its relatively higher field chemical shift. The ^{13}C chemical shifts of C-6 and C-7 were similar to those of the corresponding carbons in citrinin (**4**).[8,9] The phenolic hydroxyl proton on C-6 appeared as a sharp singlet signal, due to initramolecular hydrogen bonding between the hydroxyl hydrogen atom of C-6 and the carbonyl oxygen atom of C-12. The ether linkage between C-8 and C-2′ was suggested by the ROESY correlation for OH-12/H-2′ observed at 0 °C. Therefore, the gross structure of perinadine A was assigned as **1**.

The relative stereochemistry of the tetracyclic core in perinadine A (**1**) was elucidated on the basis of NOESY data and $^1H-^1H$ coupling constants (Figure 2). The $^1H-^1H$ coupling constant for H-3−H-4 (7.2 Hz) was suggested to be a diaxial orientation for H-3 and H-4. Since a NOESY correlation was observed for H-1/H-3, H-1 was considered to have an axial orientation. The anti relationship between H-1 and H-3′ was deduced from NOESY correlations for H-1/H-4′β (δ_H 2.26), H_3-9/H-3′, and H-3′/H-4′α (δ_H 2.46), while the NOESY correlation for H-2′/H-3′ indicated the cis relationship between H-2′ and H-3′. Therefore, the five sp^3 methine protons in **1** were concluded to have 1β, 3β, 4α, 2′α, and 3′α orientations. The CD spectrum of perinadine A (**1**) showed exciton maxima at 313 ($\Delta\epsilon$ −0.5), 257 (+1.3), 224 (−0.6), and 214 nm (+0.6), which were similar to the Cotton curve [λ_{ext} 307 ($\Delta\epsilon$ −0.2), 252 (+1.0), 230 (−0.5),

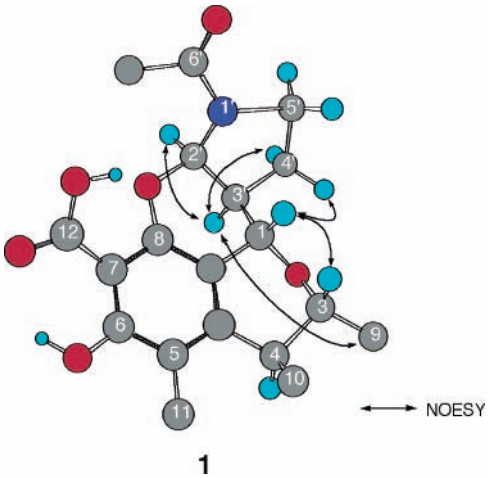

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Figure 2. Selected NOESY correlations and relative stereochemistry of the tetracyclic core in perinadine A (**1**). $^1H-^1H$ coupling constants (H/H): H-1/H-3′, 4.8 Hz; H-3/H-4, 7.2 Hz; H-2′/H-3′, 5.4 Hz.

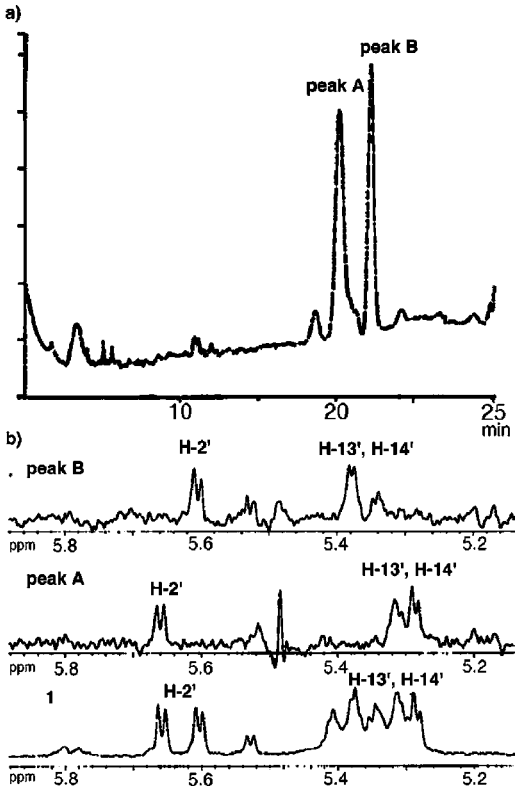

Figure 3. LC NMR analysis of perinadine A (**1**). (a) HPLC chromatography of perinadine A (**1**) and (b) part of the ^1H NMR spectra of peaks **A** and **B** and perinadine A (**1**) in $CD_3CN/D_2O/CF_3CO_2H$ (75:25.0.1).

(7) The ^{13}C chemical shift of C-15 of **1** corresponded to that of C-4 (δ_C 17.6) in *E*-1-chlorobut-2-ene rather than that in *Z*-1-chlorobut-2-ene (δ_C 12.6): Kalinowski, H.-O.; Berger, S.; Braun, S. In *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1988; pp 293−294.

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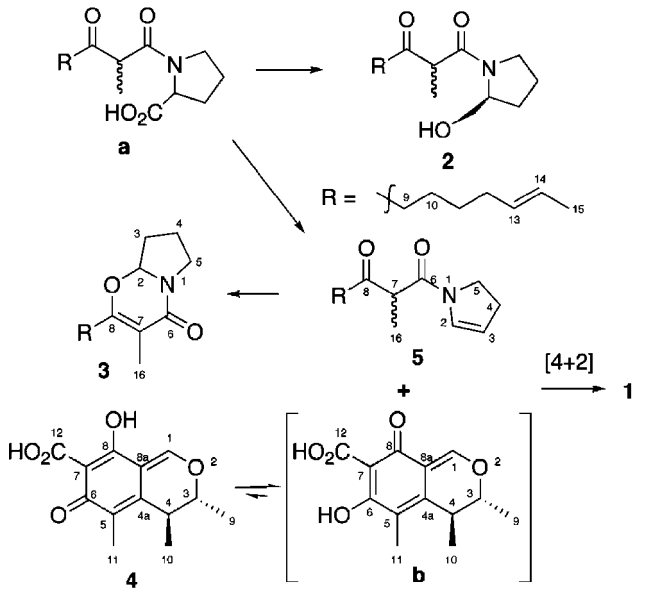
(9) ^{13}C NMR data of citrinin (**4**): Sankawa U.; Ebizuka, Y.; Noguchi, H.; Isikawa, Y.; Kitagawa, S.; Yamamoto, Y.; Kobayashi, T.; Iitaka, Y. *Tetrahedron* **1983**, *39*, 3583−3591.

Scheme 1



and 217 nm (+0.2)] of citrinin (**4**). This suggested that the three chiral centers in the dihydropyran ring of **1** possessed the same absolute configurations as those of citrinin (**4**). Therefore, the absolute configurations at C-1, C-3, C-4, C-2′, and C-3′ were assigned as *S*, *R*, *R*, *R*, and *S*, respectively. Two components of perinadine A (**1**) were examined by LC NMR analysis using C_{18} HPLC (Figure 3). The HPLC analysis of perinadine A (**1**) afforded two clearly distinguished peaks **A** (t_R 20.3 min) and **B** (t_R 22.4 min) (Figure 3a). The stopped-flow mode ^{1}H NMR spectra of peaks **A** and **B** disclosed differences in some resonances, especially H-2′ signals [peak **A**, peak **B**, and perinadine A (**1**): Figure 3b], suggesting that **1** consisted of two components corresponding to peaks **A** and **B**. Nevertheless, these two components would be converted to each other soon after separation and evaporation. In the ^{13}C NMR spectrum in MeOH-d_4, four sets of carbon resonances consisting of two strong and two weak peaks were observed, probably due to the rotational isomer of the N-1′−C-6′ amide bond.

Perinadine A (**1**) is a novel tetracyclic alkaloid with a 2-methyl-3-keto C_{10} acyl group. Biogenetically, perinadine A (**1**) may be derived from a known pyrrolidine alkaloid[3] (**5**) isolated from *Penicillium brevicompactum* and citrinin[8−10] (**4**), a well-known mycotoxin (Scheme 1). An intermediate **a**, which may be derived from glutamic acid or proline and a pentaketide, are considered to be converted into **5** by decarboxylation at C-16. Intermolecular cyclization between **5** and an intermediate **b** equal to citrinin (**4**) may give perinadine A (**1**). On the other hand, scalusamide A (**2**) may be generated from **a** through reduction of the carboxyl group at C-16, while pyrrolo[2,1-*b*]oxazines[3,4] (**3**) is likely to be converted from **5** through Michael-type cyclization between C-2 and a carbonyl oxygen at C-8.

Perinadine A (**1**) showed weak cytotoxicity against murine leukemia L1210 cells (IC_{50}, 20 μg/mL) and antibacterial activity against *Micrococcus luteus* (MIC, 33.3 μg/mL) and *Bacillus subtilis* (MIC, 66.7 μg/mL).

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Supporting Information Available: Experimental procedures and spectral data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Citrinin (**4**) has not been isolated from the extract of a rotary-shaking cultivation of this strain (N055) of *P. citrinum*. For another strain (N059) of *P. citrinum*, citrinin (**4**) was observed as a major component in the culture supernatant by stationary cultivation, while this strain produced **4** in rotary-shaking cultivation.