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Citrinadin A, a Novel Pentacyclic Alkaloid from Marine-Derived Fungus *Penicillium citrinum*

Masashi Tsuda,[†] Yuu Kasai,[†] Kazusei Komatsu,[†] Teruo Sone,[‡] Michiko Tanaka,[‡] Yuzuru Mikami,[§] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan

jkobay@pharm.hokudai.ac.jp

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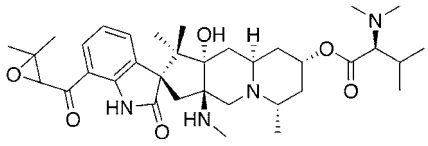
ABSTRACT

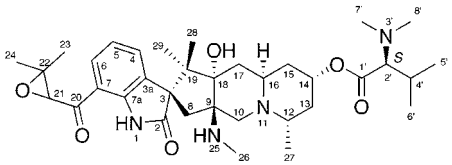


citrinadin A (**1**)

A novel pentacyclic alkaloid, citrinadin A (1), was isolated from the cultured broth of the fungus *Penicillium citrinum*, which was separated from a marine red alga, and the structure was elucidated by spectroscopic data. The relative stereochemistry of the pentacyclic core was assigned on the basis of NOESY data and ^{1}H–^{1}H coupling constants, and the presence of an *N*,*N*-dimethyl-L-valine residue in 1 was determined by chiral HPLC analysis of the hydrolysate.

Marine-derived fungi 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] a novel pentacyclic spiroindolinone alkaloid, citrinadin A (**1**), with an *N*,*N*-dimethylvaline residue and an α,β-epoxy carbonyl unit, was isolated from the cultured broth of a fungus *Penicillium citrinum*, which was separated from a marine red alga. In this paper, we describe the isolation and structure elucidation of **1**.

The fungus *Penicillium citrinum* (strain N-059) was separated from the red alga *Actinotrichia fragilis* collected at Hedo Cape, Okinawa Island, and grown in PYG liquid medium containing seawater for 14 days at 25 °C. The mycerium (435 g) of the culture broth (12 L) was extracted with MeOH. The extract was partitioned between hexane and 90% aqueous MeOH, and the MeOH-soluble portion was extracted with *n*-BuOH. The *n*-BuOH-soluble portions were subjected to LH-20 and SiO_2 column chromatographies to afford the bis-salt of citrinadin A (**1**, 6.5 mg, 0.0015%, wet weight).



1

The bis-salt of citrinadin A[3] {**1**, $[\alpha]^{19}_D$ −17° (*c* 0.4, MeOH)} showed the pseudomolecular ion peak at *m*/*z* 625 in the FABMS, and the molecular formula was revealed to be $C_{35}H_{52}O_6N_4$ by HRFABMS [*m*/*z* 625.3964, $(M + H)^+$,

* To whom correspondence should be addressed. Phone: +81-11-706-4985. Fax: +81-11-706-4989.
† Graduate School of Pharmaceutical Sciences, Hokkaido University.
‡ Graduate School of Agriculture, Hokkaido University.
§ Chiba University.

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Table 1. ^{1}H and ^{13}C NMR Data of the Bis-Salt of Citrinadin A (**1**) in $CDCl_3$

position	δ_C	m		δ_H	m, Hz
1				9.64	s
2	184.89	s			
3	60.42	s			
3a	134.52	s			
4	133.30	d		7.66	d, 7.4
5	122.49	d		7.19	t, 7.7
6	127.64	d		7.78	d, 8.0
7	117.52	s			
7a	142.67	s			
8	41.52	t		2.20[a]	s
9	67.90	s			
10	51.31	t	(α)	3.26	d, 11.4
			(*β*)	3.81	m
11				11.27	brs
12	56.12	d		3.69	m
13	32.70	t	(α)	1.88	brd, 16.0
			(*β*)	3.47	m
14	68.92	d		5.43	brs
15	33.34	t	(α)	2.00	brd, 14.6
			(*β*)	3.13	brt, 13.8
16	47.48	d		4.07	m
17	31.35	t	(α)	1.80	brd, 12.0
			(*β*)	2.17	brt, 12.0
18	82.13	s			
18-OH				5.38	brs
19	51.00	s			
20	194.84	s			
21	64.06	d		4.05	s
22	61.72	s			
23	24.31	q		1.60[b]	s
24	18.62	q		1.37[b]	s
25				2.75	br
26	30.57	q		2.50[b]	s
27	15.21	q		1.59[b]	d, 6.4
28	21.93	q		1.02[b]	s
29	27.96	q		1.37[b]	s
1′	166.72	s			
2′	71.38	d		3.77	d, 6.7
3′				12.68	brs
4′	28.35	d		2.40	m
5′	19.47	q		1.07[b]	d, 6.5
6′	21.37	q		1.35[b]	d, 6.5
7′	38.34	q		2.99[b]	brs
8′	43.14	q		2.94[b]	brs

[a] 2H. [b] 3H.

−0.1 mmu]. The IR spectrum suggested the presence of OH/NH (3401 cm^{-1}) and carbonyl group(s) (1710 and 1671 cm^{-1}). The UV absorption at 335 nm (ϵ 3100) was attributed to a conjugated benzenoid chromophore. The ^{13}C NMR (Table 1) spectrum disclosed the existence of three carbonyls (δ_C 194.84, 184.89, and 166.72), three sp^2 quaternary carbons (δ_C 142.64, 134.52, and 117.52), three sp^2 methines (δ_C 133.30, 127.64, and 122.49), five sp^3 quaternary carbons (δ_C 82.13, 67.90, 61.72, 60.42, and 51.00), six sp^3 methines (δ_C 71.38, 68.92, 64.06, 56.12, 47.48, and 28.35), five sp^3 methylene (δ_C 51.31, 41.52, 33.34, 32.70, and 31.35), and 10 methyls (δ_C 43.14, 38.34, 30.57, 27.96, 24.31, 21.93, 21.37, 19.47, 18.62, and 15.21). Because six out of 12 unsaturations were accounted for, **1** was inferred to contain six rings.

The ^{1}H NMR (Table 1) spectrum included five D_2O-exchangeable proton signals (δ_H 12.68, 11.27, 9.64, 5.38, and 2.75) due to NH and/or OH groups. One (δ_H 11.27, NH-11) of two low-field D_2O-exchangeable resonances showed cross-peaks to H-10*β* (δ_H 3.81), H-12 (δ_H 3.69), and H-16 (δ_H 4.07) in the ^{1}H−^{1}H COSY spectrum, whereas the other (δ_H 12.68, NH-1) correlated with a methine (δ_H 3.77, H-2′) and two *N*-methyl signals (δ_H 2.99, H_3-7′; δ_H 2.94, H_3-8′) in the ^{1}H−^{1}H COSY and the TOCSY spectra. These observations suggested that the two resonances were attributed to ammonium NH signals at N-11 and N-3′. The ^{1}H−^{1}H COSY, TOCSY, and HSQC spectra revealed four connectivities from C-4 to C-6, from C-10 to C-17 and C-27 through N-11, from NH-25 to C-26, and from C-4′ to C-8′ through N-3′ and C-2′ (Figure 1). The existence of a 1,2,3-

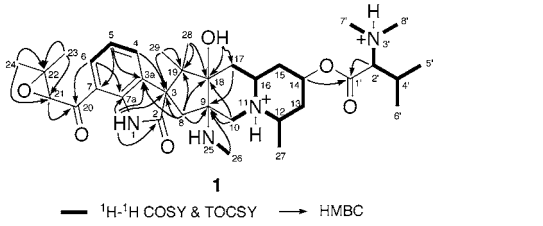

Figure 1. Selected 2D NMR correlations for citrinadin A (**1**).

trisubstituted benzene ring was indicated by HMBC correlations for H-4 (δ_H 7.66)/C-3, H-4/C-7a (δ_C 142.67), H-5 (δ_H 7.19)/C-3a (δ_C 134.52), H-5/C-7 (δ_C 117.52), and H-6 (δ_H 7.78)/C-7a. HMBC correlations were observed for NH-1 (δ_H 9.64)/C-2 (δ_C 184.89), NH-1/C-3 (δ_C 60.42), NH-1/C-3a, NH-1/C-7a, and H-4/C-3, suggesting that **1** possessed an indolinone ring (C-1−C-7a). The presence of 2,3-epoxy-3-methyl-1-oxobutyl side chain (C-20−C-24) at C-7 was deduced from HMBC correlations for H-7/C-20 (δ_C 194.84), H-21 (δ_H 4.05)/C-20, H_3-23 (δ_H 1.60)/C-21 (δ_C 64.06), H_3-23/C-22 (δ_C 61.72), H_3-24 (δ_H 1.37)/C-21, and H_3-24/C-22. ^{1}H NMR data of this C_5 unit in **1** were similar to those of the corresponding part of hopeyhopin.[4] Long-range H−C couplings for H-10α (δ_H 3.26)/C-9 (δ_C 67.90), H-10α/C-18 (δ_C 82.13), and H-17*β* (δ_H 2.17)/C-9 suggested the presence of a quinolizidine ring system (N-11 and C-9−C-18). HMBC correlations for a D_2O-exchangeable proton (OH-18) at δ_H 5.38 to C-17 and C-18 indicated that a hydroxyl group was attached to C-18. It was revealed that an *N*-methylamino group (N-25−C-26) was connected to C-9 since an HMBC

(3) **Bis-salt of citrinadin A (1):** colorless oil; UV (MeOH) λ_{max} 335 (ϵ 3100), 265 (sh.), 249 (9600), 230 (sh.), and 224 (9000); IR (neat) ν_{max} 3401, 2930, 1710, 1671, and 1602 cm^{-1}; FABMS *m*/*z* 625 $(M + H)^+$; HRFABMS *m*/*z* 625.3964 [$(M + H)^+$, calcd for $C_{35}H_{53}O_6N_4$, 625.3965].

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correlation was observed for H_3-26 (δ_H 2.50)/C-9. In the HMBC spectrum, a singlet proton signal at δ_H 2.20 (2H, H_2-8) showed correlations to C-9, C-18, and C-19 (δ_C 51.00), and both of two singlet methyl signals at δ_H 1.02 (H_3-28) and 1.37 (H_3-29) were correlated to C-18 and C-19, suggesting the presence of a cyclopenta[*b*]quinolizidine moiety (N-11, C-3, and C-8−C-19). HMBC correlations for H_2-8/C-3, H_2-8/C-3a, and H_3-29/C-3 indicated that the cyclopenta[*b*]quinolizidine moiety and the indolinone ring were connected to each other through the spiro carbon (C-3). The presence of an *N*,*N*-dimethylvaline residue was deduced from the HMBC correlation for H-2′/C-1′ (δ_C 166.72). The ^{13}C chemical shift at C-1′ were close to those of the *N*,*N*-dimethylvaline ester in 14-(*N*,*N*-dimethyl-L-valyloxy)paspalinine[5] rather than those of the *N*,*N*-dimethylvalinamide terminus in dolastatin 10,[6] indicating that **1** possessed an *N*,*N*-dimethylvaline ester. The relatively low-field chemical shift of H-14 (δ_H 5.43) suggested that the *N*,*N*-dimethylvaline residue was attached to C-14 via an ester linkage.[7] [This was supported by high-field shift of H-14 by hydrolysis of the *N*,*N*-dimethylvalyl ester (vide infra).] Therefore, the gross structure of citrinadin A was concluded to be **1**.

The relative stereochemistry of the pentacyclic core of citrinadin A (**1**) was elucidated on the basis of ROESY data and ^{1}H−^{1}H coupling constants (Figure 2). ROESY correlations for H-4/H_3-26 and H-4/H_3-29 indicated that one (C-29) of two methyl groups at C-19, the C-3−C-3a bond, and the methylamino group (C-25−C-26) were β-oriented. Axial orientations for the β-protons at C-13, C-15, and C-17 were implied from ROESY correlations for H-13β/H-15β, H-15β/H-17β, and H-17β/H_3-29 and proton signal patterns for H-15β (brt, J = 13.8 Hz) and H-17β (brt, J = 12.0 Hz). On the other hand, ROESY correlations for H-10α/H_3-27, H-10α/OH-18, H-16/OH-18, H-16/H_3-27, and OH-18/H_3-28 suggested α-axial orientations for H-10α, H-16, OH-18, and H_3-27. Because ROESY correlations were observed for H-10β/H-12, H-15α/H-17α, and H-17α/H_3-28, H-10b, H-12, H-15a, and H-17a were considered to have equatorial orientations. The broad singlet pattern of H-14 suggested that the oxygen atom at C-14 was α-axially oriented. Therefore, the relative configuration of the cyclopenta[*b*]quinolizidine moiety in **1** was elucidated to be anti/syn/anti and chair forms for two six-membered rings. On the other hand, it was difficult to elucidate unambiguously the relative configuration of the epoxide ring at C-21−C-22.

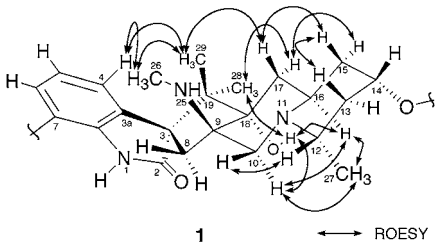

Figure 2. Relative stereochemistry for the pentacylic ring core of citrinadin A (**1**).

The absolute configuration at C-2′ in the *N*,*N*-dimethylvaline residue was determined on the basis of chiral HPLC analysis of the hydrolysate of citrinadin A (**1**). Hydrolysis of **1** with 1 N aqueous HCl afforded 1 mol of *N*,*N*-dimethylvaline. The *N*,*N*-dimethylvaline residue was identified as L-form by chiral HPLC analysis using authentic D- and L-*N*,*N*-dimethylvaline.[8]

Citrinadin A (**1**) is a novel pentacyclic spiroindolinone alkaloid with an *N*,*N*-dimethylvaline ester and an α,β-epoxy carbonyl unit. There are only two prior reports of the natural occurrence of the *N*,*N*-dimethylvaline residue describeed in 14-(*N*,*N*-dimethyl-L-valyloxy)paspaline[5] and dolastatins,[6] which were isolated from fungi and sea hares, respectively. Although several spiroindolinone alkaloids such as brevianamides,[9] paraherquamides,[10] marcfortines,[11] sclerotamide,[12] and asperparalins[13] have been isolated from fungi of the genera *Penicillium* or *Aspergillus*, the pentacylcic skeleton such as **1** is unique. Citrinadin A (**1**) exhibited cytotoxicity against murine leukemia L1210 and human epidermoid carcinoma KB cells (IC_{50} 6.2 and 10 μg/mL, respectively).

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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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