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Unusual C25 Steroids Produced by a Sponge-Derived *Penicillium citrinum*

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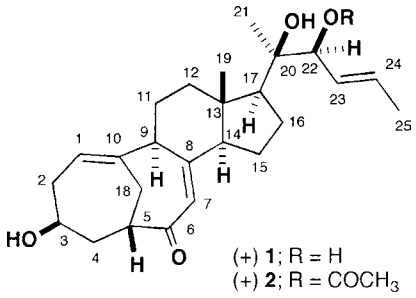
ABSTRACT



Structurally unique steroids, isocyclocitrinol A (1) and 22-acetylisocyclocitrinol A (2), were isolated from the extract of a saltwater culture of sponge-derived *Penicillium citrinum*. The structures were established by analysis of 1D and 2D NMR data. The absolute structures were determined on the basis of X-ray structure analysis and application of modified Mosher's method. Furthermore, the structure of cyclocitrinol (3a) previously isolated from a terrestrial *P. citrinum* was revised as 3b. Compounds 1 and 2 showed weak antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus durans*.

Filamentous fungi isolated from marine invertebrates are a fertile source of bioorganic compounds,[1] and therefore, interesting secondary metabolites from sponge-derived fungi are continually being found. Two diverse examples are asperazine,[2] an unusual diketopiperazine dimer isolated from a sponge-derived *Aspergillus niger*, and epoxysorbicillinol,[3] derived from a sponge-derived *Trichoderma longibrachiatum*. Recently, we have obtained a *Penicillium citrinum* strain from an Axinellida sponge, which shows selectivity against solid tumor cells in a soft agar-based bioassay system.[4] The *Penicillium* genus is one of the most ubiquitous terrestrial fungi and has been intensely studied since the discovery of the penicillins. Nevertheless, *Penicillium* spp. isolated from marine environments produce bioactive and structurally

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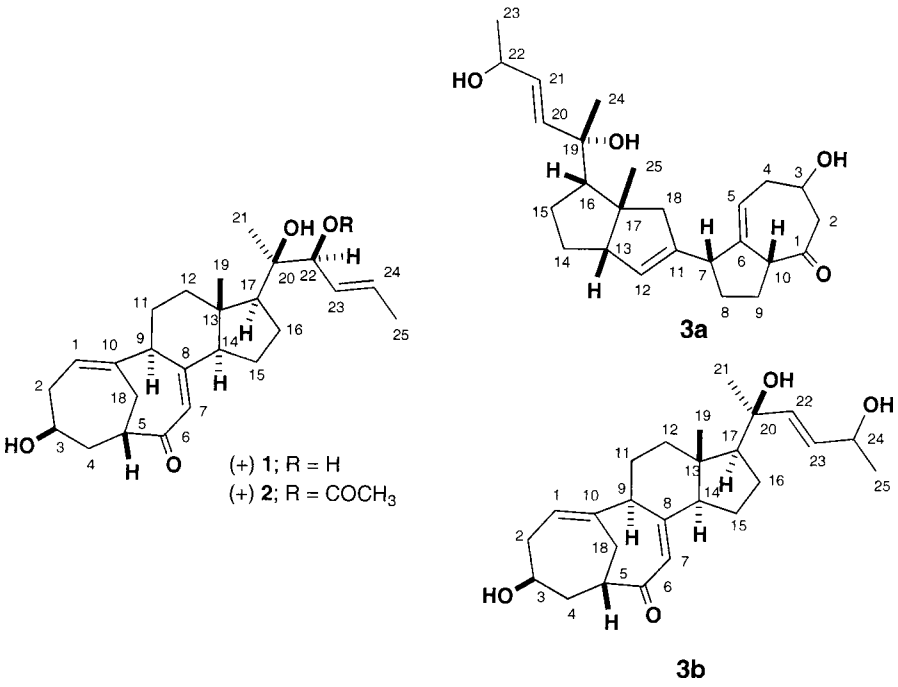

Figure 1. The structures of isocyclocitrinol A (**1**) and 22-acetylisocyclocitrinol A (**2**) along with that previously reported (**3a**) and revised (**3b**) herein for cyclocitrinol.

fascinating secondary metabolites that are not typically seen in terrestrial sources. Examples include the penostatins,[5] penochalasins,[6] and communesins.[7,8] Our recent investigation of secondary metabolites of a marine isolate of *Penicillium citrinum* led to the discovery of isocyclocitrinol A (**1**) and 22-acetylisocyclocitrinol A (**2**), novel rearranged steroids, isolated from the culture broth extract. We report the details of their structure elucidations along with a structure revision of cyclocitrinol (**3a**) isolated from a terrestrial *P. citrinum* (Figure 1).[9]

The fungus, *P. citrinum* (coll. no. 991084) was separated from a sponge (coll. no. 99108; *Axinella* sp. order Axinellida) collected in Papua New Guinea. The fungus was grown in a liquid medium (20 L) containing 3.5% Czapek-Dox broth and 0.5% yeast extract in filtered Monterey Bay seawater adjusted to pH 7.4 at 180 rpm for 28 days at room temperature (25 °C). The MeOH-soluble material of the culture broth EtOAc extract was purified by reversed-phase HPLC to afford isocyclocitrinol A (**1**) and 22-acetylisocyclocitrinol A (**2**).

The molecular formula of isocyclocitrinol A (**1**) for $C_{25}H_{36}O_5$ was established from HRESI-TOF-MS data. Dereplication employed two pieces of data as input, the molecular formula and the taxonomy *Penicillium* sp. These resulted in one hit, cyclocitrinol (**3a**), which was isolated from a terrestrial *P. citrinum* and reported as a new sesterterpenoid.[10] The ^{13}C NMR data of **1** were very similar to that of **3a** except for the side chain (Table S1). Therefore, **1** was first assumed to be a new cyclocitrinol analogue possessing a different side chain. On the basis of this first comparison, we began to elucidate the structure of **1**. A detailed inspection of the ^{1}H and ^{13}C NMR data (Table 1) by DEPT, gCOSY, and gHMQC disclosed the existence of three methyls (C19, C21, and C25) including two sp^3 singlet methyls and one olefinic methyl, seven methylenes (C2, C4, C11, C12, C15, C16, and C18), two trisubstituted and one *trans* disubstituted double bonds (C1, C7, C8, C10, C23, and C24), six methines including four sp^3 methines (C5, C9, C14, and C17) and two sp^3 methines (C3 and C22) linked to an oxygen atom, two sp^3 quaternary carbons (C13 and C20), and one carbonyl carbon. An analysis of gCOSY and gHMBC data led to three substructures (Figure 2). Substructure A (C1−C6, C10, and C18) was assembled on the basis of gCOSY correlations (C1−C5−C18) and gHMBC correlations (H1/C10, H5/C6, and H18/C1, C6, C10). Substructure B (C7−C17 and C19)

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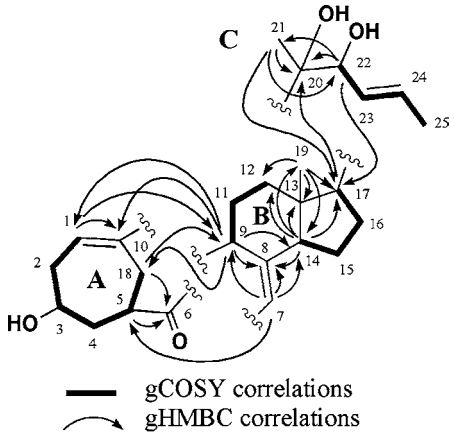

Figure 2. Substructures and selected 2D NMR correlations for **1**.

was obtained on the basis of two spin systems (C9−C11−C12 and C15−C17) and gHMBC correlations (H7/C8, C9, C14, H9/C8, C14, H14/C8, C12, C13, C17, C19, H19/C12, C13, C14, C17). Substructure C corresponding to the side chain (C20−C25) was established by gCOSY correlations (C22−C25) and gHMBC correlations (H21/C21, C22, H22/C20, C21). At this point, the task of assembling the three substructures into a unique structure was complicated because of the incompatibility of A and B with **3a**. The substructures A and B were not present in **3a**, nor were alternative proposals for A and B. However, a plausible structure could be envisioned on the basis of the connections among the substructures shown in Figure 2. The structure of **1** was firmly established by X-ray crystallographic analysis of 22-acetylisocyclocitrinol A (**2**), whose properties are discussed below. Contrary to our expectation, the structure of **1** turned out to be completely different from that of **3a** and consists of a unique four-ring system including two seven-membered rings possessing a double bond on the bridgehead. Its carbon skeleton is unprecedented. The absolute stereostructure of **1** was determined by agreement of all spectral data between **1** and the hydrolysis product of **2**, of which the absolute stereostrucure was elucidated as described below.

The second isolate, 22-acetylisocyclocitrinol A (**2**), had the molecular formula $C_{27}H_{38}O_5$ data established by HRESI-TOF-MS. No natural product possessing this molecular formula has been isolated from *Penicillum* sp. The ^{1}H and ^{13}C NMR spectra (Table S2) were similar to those of **1;** in

Table 1. NMR Data of Isocyclocitrinol A (**1**) and 22-Acetylisocyclocitrinol A (**2**) in $CDCl_3$[a]

	1				**2**			
position	δ_H (J in Hz)		δ_C		δ_H (J in Hz)		δ_C	
1	5.57	dd (8.5, 6.1)	121.9	(d)	5.57	dd (7.8, 6.4)	122.0	(d)
2α	2.25	ddt (13.3, 8.5, 2.3)	35.7	(t)	2.25	ddt (13.3, 8.2, 2.4)	35.7	(t)
β	2.49	ddd (13.3, 11.3, 6.1)			2.49	ddd (13.3, 11.4, 6.2)		
3	3.51	tdd (11.3, 4.3, 2.3)	64.6	(d)	3.51	tdd (11.4, 4.1, 2.4)	64.5	(d)
4α	2.90	brd (13.1)	41.7	(t)	2.90	brd (12.9)	41.7	(t)
β	1.68	ddd (13.1, 11.3, 4.3)			1.68	ddd (12.9, 10.8, 4.3)		
5	2.75	m	48.5	(d)	2.75	m	48.5	(d)
6			205.1	(s)			205.1	(s)
7	5.60	s	125.2	(d)	5.59	s	125.2	(d)
8			157.3	(s)			157.0	(s)
9	2.78	dd (12.5, 5.9)	54.0	(d)	2.78	dd (12.4, 5.7)	53.9	(d)
10			146.0	(s)			145.9	(s)
11α	1.61	m	27.7	(t)	1.61	m	27.6	(t)
β	1.86	m			1.86	m		
12α	1.51	m	39.4	(t)	1.48	m	39.4	(t)
β	2.17	ddd (13.0, 4.5, 2.5)			2.17	ddd (12.7, 4.5, 2.5)		
13			46.4	(s)			46.2	(s)
14	2.13	ddd (12.3, 6.9, 2.0)	55.8	(d)	2.11	ddd (12.3, 6.4, 1.8)	56.0	(d)
15α	1.63	m	22.8	(t)	1.62	m	22.6	(t)
β	1.55	m			1.54	m		
16α	1.91	m	21.8	(t)	1.90	m	21.6	(t)
β	1.81	m			1.77	m		
17	1.95	t (9.6)	54.7	(d)	1.77	t (9.6)	54.9	(d)
18α	2.55	d (13.6)	27.6	(t)	2.55	d (13.7)	27.6	(t)
β	2.60	dd (13.6, 6.2)			2.59	dd (13.7, 6.2)		
19	0.86	s	14.0	(q)	2.59	dd (13.7, 6.2)		
20			75.7	(s)			74.8	(s)
21	1.28	s	23.0	(q)	1.31	s	23.1	(q)
22	3.87	d (7.4)	79.8	(d)	5.08	d (7.5)	79.6	(d)
23	5.57	ddq (15.4, 7.4, 1.9)	129.4	(d)	5.45	ddq (15.3, 7.5, 1.6)	125.0	(d)
24	5.77	dqd (15.4, 6.4, 1.0)	130.4	(d)	5.81	dqd (15.3, 6.6, 0.9)	132.8	(d)
25	1.76	dd (6.4, 1.9)	18.0	(q)	1.75	dd (6.6, 1.6)	18.1	(q)
3-OH	nd				nd			
22-OH	nd							
$COCH_3$							170.0	(s)
$COCH_3$					2.08	s	21.3	(q)

[a] Measured at 500 MHz (^{1}H) and 125.7 MHz (^{13}C).

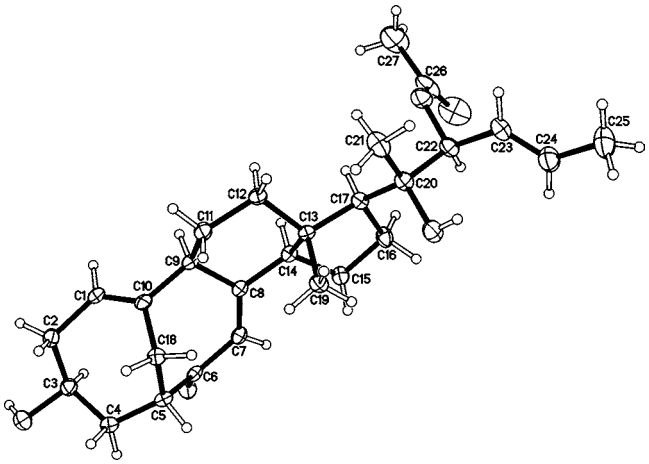

Figure 3. X-ray crystal structure for **2**.

addition an acetyl group (δ_H 2.08, δ_C 21.3 and 170.0) was observed. Therefore, the structure of **2** was expected to be an acetate of isocyclocitrinol A (**1**). Since **2** was obtained as crystals from MeOH solution, an X-ray crystal structure analysis was carried out.[11] The result (Figure 3) gave the gross structure of **2** and allowed assignment of the relative configurations of all chiral centers. The absolute stereostructure was determined by an application of modified Mosher's method (Figure 4).[12]

The last aspect of our study was to reconsider the structure of cyclocitrinol (**3a**). Side by side comparison of ^{13}C NMR data between **3** and **1** (Table S1) indicated that the A/B/C/D rings were identical with those of **1**. Considering that the side chain of **3** was different from that of **1**, the structure of **3a** was revised as **3b** shown in Figure 1.

Isocyclocitrinols A (**1**) and 22-acetylisocyclocitrinol A (**2**) are inactive against murine and human tumor cell lines in a disk diffusion assay. However, **1** and **2** show weak antibacterial activity against *Staphylococcus epidermidis* (MIC = 100 μg/mL, each) and *Enterococcus durans* (MIC = 100 μg/mL, each).

(11) Crystallographic data for 22-acetylisocyclocitrinol A (**2**) (deposition number 220617) have been deposited at the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax +44-(0)1223-336033; e-mail deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk/].

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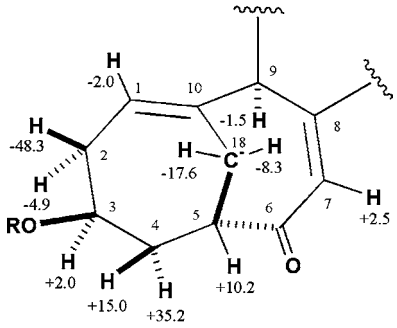

4a; R = (*R*)-MTPA
4b; R = (*S*)-MTPA

Figure 4. Proton chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters **4a** and **4b** of 22-acetylisocyclocitrinol A (**2**), expressed in Hz (500 MHz).

The citrinane framework present in **1**, **2**, and **3b** is unique and represents the first example of a steroid possessing the bicyclo [4.4.1] A/B ring system. Biogenetically this framework most likely arises via a 1,2 migration of the C5−C10 bond to give a new C5−C18 bond. Continuing work in our laboratory on additional minor constituents of the culture broth should provide additional insights about the intermediates involved in this interesting rearrangement.

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Supporting Information Available: Experimental section, ^{1}H and ^{13}C NMR spectra of **1** and **2**, and X-ray data of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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