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BRIEF COMMUNICATION

New tetrahydroquinoline and indoline compounds containing a hydroxy cyclopentenone, virantmycin B and C, produced by *Streptomyces* sp. AM-2504

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Abstract

Two new antibiotics, designated virantmycin B (**1**) and C (**2**), were isolated from the cultured broth of *Streptomyces* sp. AM-2504. Compounds **1** and **2** were purified by Diaion HP-20, silica gel, and octadecylsilane chromatography, followed by high-performance liquid chromatography. The chemical structures of the new compounds, **1** and **2**, were determined by nuclear magnetic resonance and mass spectrometry, as containing a terahydroquinoline and an indoline, respectively, each also containing a hydroxy cyclopentenone moiety. Both compounds demonstrated weak antimicrobial (both antibacterial and antifungal) activity and compound **1** also showed antiviral activity against the dengue virus, whereas compound **2** exhibited no antiviral properties.

We have recently discovered new secondary metabolites from actinomycetes using a screening process that is guided simply by detecting the physico-chemical properties of the compounds [1]. During our physicochemical screening program using culture broths of the Kitasato Microbial Library [2, 3], two new compounds, designated virantmycin B and C, were found in a cultured broth of *Streptomyces* sp. AM-2504 [4, 5]. The organism was first identified as being a producer of dityromycin, a peptide antibiotic, and had since been preserved for 40 years.

Virantmycin was discovered from *Streptomyces nitrosporeus* AM-2722 and exhibited potent inhibitory activity against various RNA and DNA viruses [6, 7]. The molecular structure of virantmycin was confirmed and found to contain a tetrahydroquinoline unit [8]. After this discovery of virantmycin, its derivatives, benzastatin [9, 10], JBIR-63 and JBIR-73 [11], and A-503451s [12], were also found in other *Streptomyces* species. The derivatives related to virantmycin possessed various bioactive properties, such as radical-scavenging [9], neuronal cell-protecting [10] and hypoxia-inducible factor-activating properties [12].

This paper describes the fermentation, isolation, structure determination, and some bioactive properties of the two new compounds, virantmycin B (**1**) and C (**2**).

A 1 mL of broth containing the strain AM-2504 was inoculated into 100 mL of the seed medium, consisting of starch 2.4% (Wako Pure Chemical Industries, Osaka, Japan), glucose 0.1%, peptone 0.3% (Kyokuto Pharmaceutical Industrial, Tokyo, Japan), meat extract 0.3% (Kyokuto), yeast extract 0.5% (Oriental Yeast, Tokyo, Japan), and $CaCO_3$ 0.4% in a 500-mL Erlenmeyer flask. The flasks were incubated on a rotary shaker (210 rpm) at 27 °C for 3 days. Then, 200 mL of the seed culture was transferred into a 30-L jar fermenter containing 20 L of a production medium, consisting of glucose 0.5%, corn steep powder 0.5% (Iwaki, Tokyo, Japan), oatmeal 1.0% (Nippon Food Manufacture, Hokkaido, Japan), pharmamedia 1.0% (Iwaki), K_2HPO_4 0.5%, $MgSO_4 \cdot 7H_2O$ 0.4%, and trace

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Table 1 ^{1}H and ^{13}C NMR chemical shifts of **1** and **2** in $CDCl_3$

Position	1			2		
	δ_c (ppm)	Mult.	δ_H (ppm), int., mult (*J* in Hz)	δ_c (ppm)	Mult.	δ_H (ppm), int., mult (*J* in Hz)
1	119.4	C		120.4	C	
2	130.4	CH	7.57, 1H, d, 2.0	124.0	CH	7.56, 1H, d, 1.6
3	117.6	C		128.8	C	
4	146.9	C		155.4	C	
5	113.8	CH	6.53, 1H, d, 8.4	107.7	CH	6.57, 1H, d, 8.4
6	127.3	CH	7.53, 1H, dd, 8.4, 2.0	128.6	CH	7.58, 1H, dd, 8.4, 1.6
7	166.6	C		166.8	C	
8	32.6	CH_2	H_a 3.12, 1H, dd, 16.8, 4.6	29.9	CH_2	H_a 3.08, 1H, dd, 16.0, 9.4
			H_b 2.85, 1H, dd, 16.8, 5.5			H_b 2.99, 1H, dd, 16.0, 9.4
9	67.2	CH	3.95, 1H, dd, 5.5, 4.6	66.3	CH	4.17, 1H, dd, 9.4, 9,4
10	57.6	C		73.0	C	
11	33.3	CH_2	H_a 1.82, 1H, ddd, 11.6, 11.4, 5.5	32.6	CH_2	H_a 1.56, 1H, ddd, 14.0, 12.2, 4.9
			H_b 1.52, 1H, ddd, 14.2, 13.8, 5.2			H_b 1.47, 1H, ddd, 13.8, 12.4, 5.2
12	27.7	CH_2	H_a 2.08, 1H, ddd, 13.6, 12.0, 5.6	28.1	CH_2	H_a 2.15, 1H, ddd, 12.4, 12.4, 5.2
			H_b 2.04, 1H, ddd, 13.6, 12.4, 5.8			H_b 2.01, 1H, ddd, 12.8, 12.6, 5.2
13	126.7	C		127.0	C	
14	124.7	C		124.6	C	
15	19.9	CH_3	1.62^{α}, 3H, s	20.0	CH_3	1.66^{β}, 3H, s
16	20.5	CH_3	1.62^{α}, 3H, s	20.6	CH_3	1.66^{β}, 3H, s
17	74.9	CH_2	H_a 3.66, 1H, d, 9.2	78.4	CH_2	H_a 3.53, 1H, d, 9.2
			H_b 3.50, 1H, d, 9.2			H_b 3.43, 1H, d, 9.2
18	18.4	CH_3	1.61, 3H, s	18.3	CH_3	1.64, 3H, s
19	59.5	CH_3	3.40, 3H, s	59.5	CH_3	3.42, 3H, s
7-NH			7.88, 1H, s			7.88, 1H, s
1′	115.1	C		115.1	C	
2′	172.8	C		172.7	C	
3′	25.6	CH_2	2.63, 2H, m	25.5	CH_2	2.63, 2H, m
4′	32.1	CH_2	2.55, 2H, m	32.1	CH_2	2.55, 2H, m
5′	197.5	C		197.5	C	
2′-OH			13.71, 1H, s			13.72, 1H, s

α, β: overlapped

metals solution 1 mL L^{-1} ($FeSO_4 \cdot 7H_2O$ 0.1%, $MnCl_2 \cdot 4H_2O$ 0.1%, $ZnSO_4 \cdot 7H_2O$ 0.1%, $CuSO_4 \cdot 5H_2O$ 0.1%, and $CoCl_2 \cdot 6H_2O$ 0.1%), pH 7.0, and fermentation was carried out for 7 days at 27 °C.

The isolation of **1** and **2** was guided by LC/UV (liquid chromatography with ultraviolet detection) analysis (Scheme S1). Whole cultured broth (60 L) was centrifuged for 10 min at 12,000 rpm to separate the mycelia and the supernatant. The supernatant was applied to a Diaion HP-20 (100 i.d. × 80 mm; Mitsubishi Chemical Ltd, Tokyo, Japan). After washing with 30% MeCN aq., the fraction containing **1** and **2** was eluted with 60% MeCN aq. and concentrated in vacuo to yield 11 g. This material was applied to a silica gel FL100D (65 i.d. × 90 mm; Kanto Chemical Co. Inc., Tokyo, Japan) and eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0-1, 100:0-2, 50:1-1, 50:1-2, 10:1-1, 10:1-2, 1:1-1, 1:1-2 and 0:100 (v/v), each 1.5 L), to give nine fractions. The eluate fractions (fractions 100:0-2 and 50:1-1) were concentrated in vacuo to yield 1134 mg. This material was applied to an octadecylsilane column (30 i.d. × 100 mm; Senshu Scientific, Tokyo, Japan). After washing with 40% MeCN aq., the fraction containing **1** and **2** was eluted with 60% MeCN aq. and concentrated in vacuo. The eluate fraction was dissolved in a small amount of MeOH to afford MeOH-soluble fraction (107 mg). This material was purified by high-performance liquid chromatography on an

Inertsil C8-4 column (14 i.d. × 250 mm; GL Sciences Inc., Tokyo, Japan) with 66% MeOH aq. with 0.1% formic acid at 4.7 mL min^{-1} with detection at UV 300 nm. The peaks at retention time of 23–27 and 28–32 min were collected and dried in vacuo to yield **1** (24 mg) and **2** (27 mg), respectively.

The physico-chemical properties of **1** and **2** are summarized in Table S1. The infrared absorption at 1604 and 1608 cm^{-1} suggested the presence of an amide group. Both compounds were readily soluble in MeOH and $CHCl_3$ but not in H_2O.

Compound **1** was obtained as a white powder and determined to have the molecular formula of $C_{24}H_{32}N_2O_5$ by high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS) $[M + H]^+$ ion at *m/z* 429.2395 (calculated value for $C_{24}H_{33}N_2O_5$, 429.2390) and nuclear magnetic resonance (NMR) spectral data. The ^1H and ^{13}C NMR spectral data in $CDCl_3$ of **1** are listed in Table 1. The ^1H NMR and heteronuclear single quantum coherence (HSQC) data indicated the presence of one sp^3 methine, six sp^3 methylenes, four methyls including one methoxy, and three sp^2 methines. The ^{13}C NMR spectrum and HSQC data showed the resonances of 24 carbons, which were classified into ten olefinic carbons, two carbonyl carbons, five sp^3 methylene carbons, one oxygenated sp^3 methylene carbon, three methyl carbons, one methoxy carbon, one oxygenated sp^3 methine, and one fully substituted carbon at 57.6 ppm. The ^1H-^1H COSY (COrrelated SpectroscopY) indicated the presence of four partial structures C-5/C-6, C-8/C-9, C-11/C-12, and C-3′/C-4′ as shown in Fig. 1a. Analysis of heteronuclear multiple bond correlation (HMBC) data confirmed the presence of a partial structure, including a tetrahydroquinoline moiety, based on correlations from H-2 to C-4, C-6, C-7, and C-8; from H-5 to C-1 and C-3; from H-6 to C-2, C-4, and C-7; from H_2-8 to C-2, C-3, C-4, C-9, and C-10; from H-9 to C-3, C-8, C-10, and C-17; from H_2-17 to C-9, C-10, C-11, and C-19; from H_3-19 to C-17; from H_2-11 to C-9, C-10, C-12, C-13, and C-17; from H_2-12 to C-11, C-13, C-14, and C-18; from H_3-15 to C-13, C-14, and C-16; from H_3-16 to C-13, C-14, and C-15; from H_3-18 to C-12, C-13, and C-14. The above results were supported by NMR chemical shifts reported for virantmycin [1, 2], which was reported to be the first compound found from an actinomycete strain which contained a tetrahydroquinoline moiety. The HMBC correlations from H_2-3′ to C-1′, C-2′, C-4′, and C-5′; from H_2-4′ to C-2′, C-3′, and C-5′; from 2′-OH to C-1′, C-2′, and C-3′ confirmed a hydroxy cyclopentenone moiety as a partial structure. Finally, the HMBC correlations from 7-NH to C-7, C-1′, C-2′, and C-5′ showed that the tetrahydroquinoline and the hydroxy cyclopentenone conjugated at the 7-NH position. Therefore, the structure of **1** was elucidated to be a new compound related to virantmycin, shown in Fig. 1a, and it was designated as virantmycin B (**1**).

The rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY) correlations of **1** were observed between H_b-8/H_b-11, H-9/H_a-12, and H-9/H_{ab}-17 with large coupling constants (H_b-11/H_a-12: 11.6 Hz and H_b-11/H_b-12: 12.0 Hz) (Fig. 1a). These results reveal that the relative configurations at C-9 and C-10 as R^* and R^*, respectively, and the hydroxyl group at C-9 and 2,3-dimethyl-2-pentene at C-10 is located on the opposite surface.

Compound **2** was obtained as a white powder and determined to have a molecular formula of $C_{24}H_{32}N_2O_5$ by HR-ESI-MS $[M + H]^+$ ion at *m/z* 429.2376 (calculated value for $C_{24}H_{33}N_2O_5$, 429.2390) and NMR spectral data. The ^1H and ^{13}C NMR spectral data of **2** are listed in Table 1. The ^1H NMR and HSQC data indicated the presence of one sp^3 methine, six sp^3 methylenes, four methyls including one methoxy, and three sp^2 methines. The ^{13}C NMR spectrum and HSQC data showed the resonances of 24 carbons, which were classified into ten olefinic carbons, two carbonyl carbons, six sp^3 methylene carbons, one heteroatom bonded sp^3 methine, three methyl carbons, one methoxy carbon, and one oxygenated carbon at 73.0 ppm. The ^1H-^1H COSY indicated the presence of four partial structures C-5/C-6, C-8/C-9, C-11/C-12, and C-3′/C-4′, as shown in Fig. 1b. Analysis of HMBC data confirmed the presence of a partial structure, including a indoline moiety, based on correlations from H-2 to C-4, C-6, C-7, and C-8; from H-5 to C-1 and C-3; from H-6 to C-2, C-4, and C-7; from H-8 to C-2, C-3, C-4, C-9, and C-10; from H-9 to C-8 and C-17; from H_2-17 to C-9, C-10, C-11, and C-19; from H_3-19 to C-17; from H_2-11 to C-9, C-10, C-12, and C-17; from H_2-12 to C-10, C-11, C-13, C-14, and C-18; from H_3-15 to C-13, C-14, and C-16; from H_3-16 to C-13, C-14, and C-15; from H_3-18 to C-12, C-13, and C-14. The HMBC correlations from H_2-3′ to C-1′, C-2′, C-4′, and C-5′; from H_2-4′ to C-2′, C-3′, and C-5′; from 2′-OH to C-1′, C-2′, and C-3′ confirmed a hydroxy cyclopentenone moiety as a partial structure. Finally, the HMBC correlations from 7-NH to C-7, C-1′, C-2′, and C-5′ showed that the indoline and the hydroxy cyclopentenone moieties were conjugated at 7-NH position. Therefore, the structure of **2** was elucidated as shown in Fig. 1b, and it was designated as virantmycin C (**2**).

The ROESY correlations of **2** were observed between H_a-8/H_{ab}-11, H-9/H_a-17, and H_b-12/H_b-17 with large coupling constants (H_a-11/H_a-12: 12.3 Hz and H_b-11/H_b-12: 12.5 Hz) (Fig. 1b). These results reveal that the relative configurations at C-9 and C-10 are S^* and R^*, respectively. This is the first report of natural compounds consisting of either quinoline or indoline moieties and a hydroxy cyclopentenone.

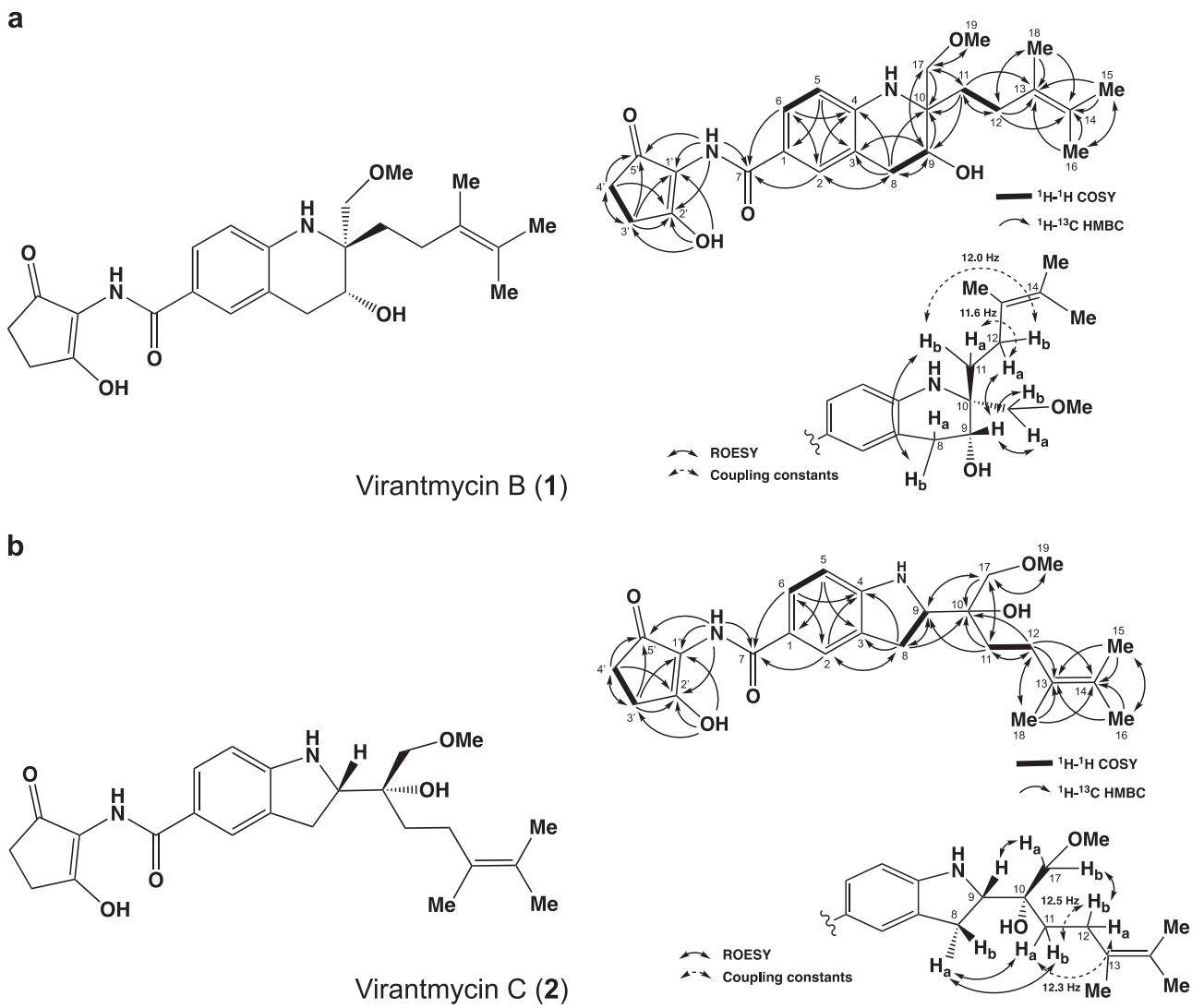

Fig. 1 NMR correlations and relative configuration of **a** virantmycin B (**1**) and **b** virantmycin C (**2**)

Compounds **1** and **2** both showed weak antimicrobial effects against *Bacillus subtilis* ATCC 6633 and *Mucor racemosus* IFO 4581 (Table S2). In addition, compound **1** inhibited the replication of dengue virus type 2, New Guinea C strain, with an half-maximal inhibitory concentration value of $43.25 \pm 5.95\,\mu M$ (standard error). However, compound **2** displayed no such antiviral activity (data not shown). It is suggested that the piperidine moiety of compound **1** is important for creation of the antiviral activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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