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Verticillin G, a New Antibacterial Compound from *Bionectra byssicola*

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Abstract A new epidithiodioxopiperazine compound named verticillin G along with the known compound verticillin D has been isolated from the mycelium of liquid fermentation cultures of a fungal strain *Bionectra byssicola* F120. The structure of verticillin G was determined on the basis of MS and NMR data. Verticillin G inhibited the growth of *Staphylococcus aureus* including methicillin-resistant and quinolone-resistant *S. aureus* with MIC (μg/ml) of 3∼10.

Keywords verticillin G, epidithiodioxopiperazine, anti-MRSA, *Bionectra byssicola*

Gram-positive eubacteria are representative of pathogenic microorganisms. Especially, *Staphylococcus aureus* is the most clinically important of these pathogens because of its exceptional virulence, stress tolerance, and capacity to accumulate antimicrobial resistances [1]. Methicillin-resistant *S. aureus* (MRSA) is known as a major nosocomial pathogen which has also developed resistance to many other antibiotics. Moreover, MRSA has been reported to acquire resistance to the last-resort antibiotic, vancomycin [2]. These facts suggest that *S. aureus* would fully acquire resistance to vancomycin in the near future. Therefore, it is increasingly important and necessary to find new classes of antimicrobials.

In the course of our screening for new antibacterials from microbial resources, a new epidithiodioxopiperazine compound named verticillin G (**1**) together with the known compound verticillin D (**2**) was isolated from the mycelium of liquid fermentation cultures of a fungal strain *Bionectra byssicola* F120 (Fig. 1). Compound **1** is a new derivative of dimeric epidithiodioxopiperazine compounds such as verticillins A∼F [3, 4], leptosins A∼C, K, K_1 and K_2 [5, 6], Sch52900 [7], chaetosin and chetracin A [8].

In this paper, we report the fermentation, isolation, structure determination and anti-MRSA activity of **1**.

Fermentation and Isolation

Fermentation was carried out in a liquid culture medium containing YPS medium (glucose 2%, yeast extract 0.2%, peptone 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, and KH_2PO_4 0.1%, pH 5.7 before sterilization). A piece of the strain F120 from a mature plate culture was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of the above sterile seed liquid medium and cultured on a rotary shaker (150 rpm) at 28°C for 3 days. For the production of active compounds, 15 ml of the seed culture was transferred into a 1000-ml Erlenmeyer flask containing 300 ml of the YPS medium, and cultivated on a rotary shaker (150 rpm) for 7 days at 28°C. After incubation, the fermented liquid cultures (73 liters) were centrifuged at 6000 rpm for 20 minutes, then only the mycelium parts were extracted with 80% acetone. The acetone extracts was concentrated *in vacuo* to an aqueous solution, which was then extracted with an equal volume of EtOAc three times. The EtOAc extract (5 g) was subjected to Silica gel (Merck Art No. 7734.9025) column chromatography followed by stepwise elution of $CHCl_3$ -

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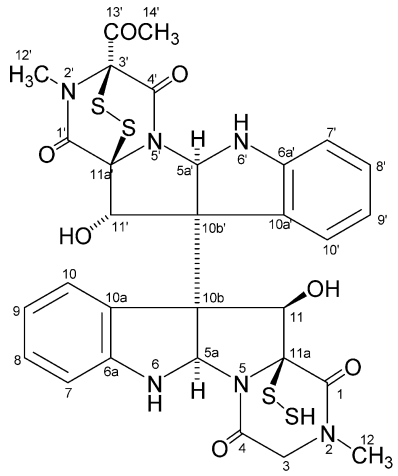

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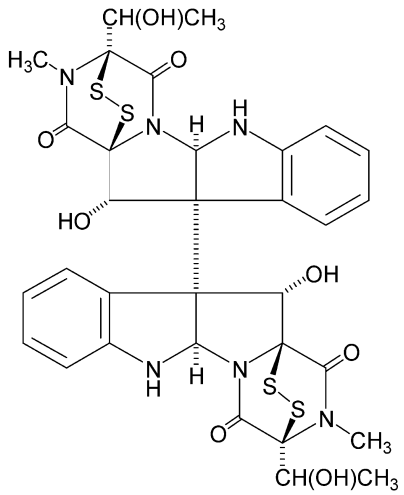

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Fig. 1 Structures of **1** and **2**.

Table 1 Physico-chemical properties of **1**

Appearance	White powder
$[\alpha]_D^{25}$	+467.6 (*c* 0.2, MeOH)
ESI-MS (*m/z*)	713 $(M+H)^+$
HRESI-MS (*m/z*)	
found.	713.09558 $(M+H)^+$
calcd.	713.09751
Molecular formula	$C_{30}H_{28}N_6O_7S_4$
UV λ_{max} nm (log ε) (MeOH)	215 (4.79), 301 (3.79)
IR ν_{max}^{KBr} cm^{-1}	3402, 2921, 1673, 1422, 1224

MeOH (100 : 1, 50 : 1). The active fractions eluted with $CHCl_3$ - MeOH (50 : 1) were pooled and concentrated *in vacuo*. The residue dissolved in MeOH was further purified by reversed-phase HPLC column (YMC C_{18}, 10×250 mm) chromatography. The column was eluted with MeOH - H_2O (75 : 25) at a flow rate of 5 ml/minute to afford **1** (2.9 mg) and **2** (35 mg) at retention times of 20.5 and 24.3 minutes, respectively.

Structure Elucidation

Physicochemical properties of **1** are shown in Table 1. The molecular formula of **1** was determined to be $C_{30}H_{28}N_6O_7S_4$ on the basis of high resolution ESI-MS [$(M+H)^+$, 713.09558 *m/z* (−1.93 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR data suggested the presence of a carboxyl (1673 cm^{-1}), and a hydroxyl (3402 cm^{-1}) moiety. The ^1H and ^{13}C NMR data (Table 2) together with ^1H-^1H COSY, DEPT and HMQC data suggested the presence of two 1,2-disubstituted benzenes, two isolated methines (δ 6.46, s, δ 76.4 and δ 7.49, s, δ 79.0) attached to two nitrogens, two isolated hydroxy methines (δ 4.35, s, δ 75.0 and δ 4.85, s, δ 84.2), an isolated methylene, an isolated methyl, two *N*-methyl groups, a ketone carbonyl, four amide carbonyls (δ 166.4, 165.8, 163.9, and 163.2), and five quaternary sp^3 carbons (δ 87.2, 85.7, 78.6, 58.3, and 56.2). These spectral data of **1** were similar to those of compound **2**, which was identified as verticillin D by comparison with the literature [3] and on the basis of various spectroscopic analyses. However, these spectral data of **1** showed a double set of signals, suggesting that **1** was not dimeric. The major differences between **1** and **2** in ^1H and ^{13}C NMR data is that the isolated methyl (δ 2.30, s, δ 25.1), the ketone carbonyl (δ 196.3), and the isolated methylene (δ 3.70, d, J=17.6 Hz and δ 4.50, d, J=17.6 Hz, δ 52.6) were newly observed in **1** instead of the signals of –CH(OH)CH_3 in **2**. Placements of the methyl, the ketone carbonyl, and the methylene were determined by HMBC spectral data (Fig. 2). The methyl protons at δ 2.30 (14′-H_3) were long range coupled to the ketone carbonyl carbon at δ 196.3 (C-13′) and the quaternary sp^3 carbon at δ 87.2 (C-3′). *N*-Methyl proton at δ 2.80 (12′-H_3) were coupled to the carbon of C-3′ and the amide carbonyl carbon at δ 166.4 (C-1′). This spectral data revealed the presence of the acetyl group which was attached to C-3′ of one epidithiodioxopiperazine ring. On the other hand, the methylene protons at δ 3.70 (3-H_a) and 4.50 (3-H_b) were long range coupled to the other *N*-methyl carbon at δ 33.8 (C-12) and two amide carbonyl carbons at δ 165.8 (C-4) and 163.2 (C-1). Also, long range couplings were also observed from the *N*-methyl protons of 12-H_3 to the amide carbonyl carbon of C-1 and the methylene carbon of C-3.

Table 2 ^{1}H and ^{13}C NMR spectral data for **1**

Position	δ_H (mult., *J* Hz)	δ_C	
1		163.2	C
3	Ha 3.70 (d, 17.6)	52.6	CH_2
	Hb 4.50 (d, 17.6)		
4		165.8	C
5a	6.46 (s)	76.4	CH
6a		148.6	C
7	6.48 (dd, 7.4, 1.0)	110.0	CH
8	6.95 (td, 7.4, 1.0)	129.4	CH
9	6.62 (td, 7.4, 1.0)	120.2	CH
10	7.36 (dd, 7.4, 1.0)	123.2	CH
10a		125.0	C
10b		58.3	C
11	4.35 (s)	75.0	CH
11a		85.7	C
12	2.95 (s)	33.8	CH_3
1′		166.4	C
3′		87.2	C
4′		163.9	C
5′a	7.49 (s)	79.0	CH
6′a		150.4	C
7′	6.34 (dd, 7.8, 1.0)	109.0	CH
8′	6.89 (td, 7.8, 1.0)	130.4	CH
9′	6.49 (td, 7.8, 1.0)	119.1	CH
10′	7.06 (dd, 7.8, 1.0)	125.8	CH
10′a		126.8	C
10′b		56.2	C
11′	4.85 (s)	84.2	CH
11′a		78.6	C
12′	2.80 (s)	30.6	CH_3
13′		196.3	C
14′	2.30 (s)	25.1	CH_3

^{1}H and ^{13}C NMR spectral data were measured at 600 and 125 MHz, respectively, in $CDCl_3$+CD_3OD. The assignments were aided by ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC.

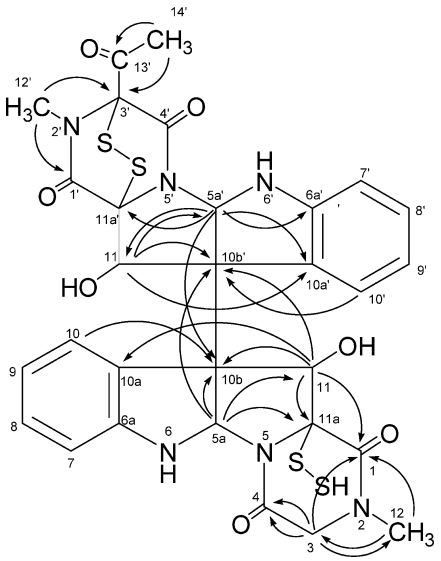

Fig. 2 Key HMBC correlations of **1**.

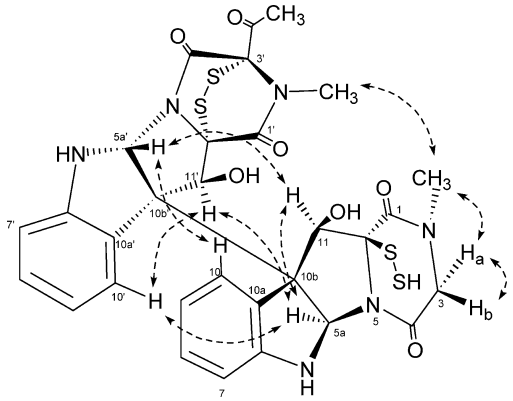

Fig. 3 Key NOE correlations of **1**.

These spectral data clearly indicated the presence of the methylene at C-3 in the other epidithiodioxopiperazine ring. Together with the molecular formula, the ^{13}C chemical shift (δ 85.7) of the quaternary sp^3 carbon of C-11a indicated that the hydrodisulfide group should be attached to C-11a in the dioxopiperazine ring. The remaining structure was also confirmed by HMBC spectral data (Fig. 2). The relative configuration of **1** was examined by NOESY spectral data (Fig. 3). Strong NOEs among 11′-H, 10′-H and 5a-H were observed, while no NOE between 11′-H and 5′a-H was observed. These data indicated that 11′-H and 5′a-H have a *trans* configuration, and 11′-H and the C-10b–C-10b′ bond have a *trans* configuration. However, 11-H showed NOEs with 5a-H and 5′a-H instead of 10-H. These data clearly indicated that 11-H and 5a-H have a *cis* configuration, and 11-H and the C-10b–C-10b′ bond have a *cis* configuration. Considering the absolute configuration of the congener **2** with the [α] value (+225°, *c* 0.1, MeOH) virtually identical with that (+220°, *c* 0.1, MeOH) of the literature [3], this data suggested the absolute configuration of **1** to be the same as that of **2** except for C-11.

1 and **2**, the dimeric epidithiodioxopiperazines, exhibited antibacterial activity against *S. aureus* (*S. aureus* RN4220 and *S. aureus* 503), MRSA (*S. aureus* CCARM 3167 and *S. aureus* CCARM 3506) and QRSA (*S. aureus* CCARM 3505 and *S. aureus* CCARM 3519). **1** showed stronger activity on QRSA with MIC (μg/ml) of 3 than against wild strains and MRSA with MIC (μg/ml) of 10. Interestingly, **2**

showed stronger activity on wild strains and MRSA with MIC (μg/ml) of 3 rather than QRSA with MIC (μg/ml) of 10. The epidithiodioxopiperazine compounds such as verticillins, leptosins and Sch52900 were known to have antibacterial and antitumor activity [3, 5]. The antitumor activity of Sch52900 was known to be due to the inhibition of *c-fos* proto-oncogene induction [7]. The active moiety of the epidithiodioxopiperazines with strong anti-MRSA activity, however, have not been reported. Thus, it is important to further investigate the structure-activity relationship of the epidithiodioxopiperazines.

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