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NOTE

Virgaricin produced by *Virgaria* sp. FKI-4860

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The genus *Virgaria* was first proposed in 1817[1] and classified in the family Xylariaceae.[2] *Virgaria nigra*, a type species of *Virgaria*, is a commonly encountered hyphomycetes and has been illustrated in numerous publications.[3–5] However, little work has been done on the chemical constituents of *Virgaria* species, and only one compound, vinigrol, has previously been isolated from *V. nigra*.[6] As part of our interest in the presence of novel metabolites in *Virgaria* sp., we investigated the chemical constituents of a culture broth of a newly discovered *Virgaria* sp. FKI-4860 that resulted in the isolation of a new compound, virgaricin (**1**). This compound was found to be an analog of pramanicins and TMC-260.[7–9] In this study, we describe the isolation, structure elucidation and antimicrobial activity of **1**.

Fungal strain FKI-4860 was isolated from a soil sample collected in the Bonin Islands, Tokyo, Japan. It is characterized by olive gray colonies, and solitary, mainly reniform and light brown sympodial conidia. The rDNA internal transcribed spacer (ITS) sequence of strain FKI-4860 was elucidated and deposited at the DNA Data Bank of Japan, with the accession number AB670709. With respect to sequence distances elucidated by the MegAlign programs from the Lasergene 9 package (DNAStar Inc., Madison, WI, USA), FKI-4860 had 90% similarity with the ITS sequences of *V. nigra* CBS 525.69 (AB670712). From the results of morphological characteristics and sequence analysis, strain FKI-4860 was identified as a novel species of *Virgaria*.[10]

Strain FKI-4860 was grown and maintained on a LcA slant consisting of 0.1% glycerol, 0.08% KH_2PO_4, 0.02% K_2HPO_4, 0.02% $MgSO_4{\cdot}7H_2O$, 0.02% KCl, 0.2% $NaNO_3$, 0.02% yeast extract and 1.5% agar (adjusted to pH 6.0 before sterilization). A loop of spores of *Virgaria* sp. FKI-4860 was inoculated into 10 ml of seed medium, which consisted of 2.0% glucose, 0.2% yeast extract, 0.5% Polypepton (Wako Pure Chemical Industries, Osaka, Japan), 0.05% $MgSO_4{\cdot}7H_2O$, 0.1% KH_2PO_4 and 0.1% agar (adjusted to pH 6.0 before sterilization), in a test tube. The inoculated tube was incubated on a rotary shaker (300 r.p.m.) at 27 °C for 3 days.

For the production of **1**, a 1-ml portion of the seed culture was transferred to each of four 500-ml Erlenmeyer flasks containing 100 ml of the production medium, consisting of 3.0% sucrose, 3.0% soluble starch, 1.0% malt extract, 0.3% Ebios (Mitsubishi Tanabe Pharma Co., Osaka, Japan), 0.5% KH_2PO_4 and 0.05% $MgSO_4{\cdot}7H_2O$ (adjusted to pH 6.0 before sterilization). Fermentation was carried out statically at 22 °C for 17 days.

The whole culture broth (400 ml) was subsequently added to an equal amount of ethanol and then filtered. The filtrate was concentrated, under reduced pressure, to remove the ethanol and then extracted with ethyl acetate. The organic layer was concentrated to dryness *in vacuo* to afford a crude extract (306 mg). The ethyl acetate extract was chromatographed on a silica gel column ($1.7\phi \times 15$ cm, Silica gel 60, Merck, Darmstadt, Germany), using a chloroform/methanol gradient solvent system of increasing the polarity, to yield four fractions. The concentrated fraction (21.5 mg) eluted with chloroform/methanol (8/2) was subjected to reversed-phase HPLC (XBridge Prep C18, Waters, Milford, MA, USA) with 40% acetonitrile at 3 ml min^{-1} detected at UV 210 nm to give **1** (3.3 mg).

Compound **1** was obtained as a colorless oil, $[\alpha]_D^{28}$ –87.3 (*c* 0.1, MeOH), UV (MeOH) λ_{max} (ε): 223 (4845), 300 (10659), 331 (14858) nm. The molecular formula of **1** was elucidated as $C_{17}H_{25}NO_5$ with six degrees of unsaturation by HR-FAB-MS (*m/z* 324.1810 $[M+H]^+$ (calculated for $C_{17}H_{26}NO_5$, 324.1811)). The IR spectrum (KBr) suggested a hydroxyl group (3411 cm^{-1}) and two carbonyl groups (1670 and 1703 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** are summarized in Table 1. The ^1H and ^{13}C NMR and HSQC spectra indicated the presence of one methyl, five methylene, eight methine, including six olefins, and three quaternary carbons (one oxygen-bearing carbon and two carbonyls). In addition, four exchangeable protons were observed in the ^1H NMR spectrum: one amide proton (δ 7.95) and three hydroxyl protons (δ 4.74, 5.61 and 6.20). ^1H-^1H COSY experiment revealed the sequences of the correlations depicted by the bold lines in Figure 1. HMBC correlations between H_2-14/C-16, H_2-15/C-16 and C-17, and H_2-16/C-17 and

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C-18 established the C-15 to C-17 connection. The large vicinal coupling constants ($J_{8,9}$=15.1, $J_{10,11}$=14.7, and $J_{12,13}$=14.9 Hz) indicated that all olefinic bonds had the *E* configuration. The ROESY correlations observed between H-8/H-10, H-9/H-11, H-10/H-12 and H-11/H-13 also supported this deduction. Furthermore, HMBC correlations from H-8 and H-9 to the carbonyl carbon at C-7 (δ 197.8) clarified the side-chain structure of (2*E*,4*E*,6*E*)-dodeca-2,4,6-trien-1-one moiety. The IR absorbance at 1670 cm^{-1} supported the presence of an α,β-unsaturated carbonyl group. The HMBC correlations of NH-1/ C-4 and C-5, OH-3/C-2, C-3 and C-4, H-4/C-3 and OH-4/C-3 suggested a 4-amino-2,3,5-trihydroxypentanoyl moiety, and the correlation between NH-1/C-3 indicated it was cyclized to form a γ-lactam ring, which was supported by the IR absorption at 1710 cm^{-1}. Finally, HMBC correlations from OH-3 and H-4 to C-7 indicated that the γ-lactam moiety was attached directly to the carbonyl carbon at C-7. On the basis of these findings, the planar structure of **1** was elucidated as shown in Figure 2.

The relative stereochemistry of **1** was elucidated by the ROESY experiment. The ROESY correlations between OH-3/H-4, OH-3/H-6a (δ 3.31) and H-4/H-6a (δ 3.31) showed that these protons were located on the same side of the molecule. The ROESY correlations between OH-4/H-8 and H-5/H-8 indicated that OH-4 and H-5 were located on the same face of the molecule. This was supported by the similarities of ^{13}C NMR data in CD_3OD (Table 1) between **1** and pramanicins,[7,8] indicating the same relative configurations at C-3, C-4 and C-5. In consequence, the relative stereochemistry of **1** in the γ-lactam moiety was assigned to be the same as for pramanicins.

Table 1 ^{1}H **and** ^{13}C **NMR data for virgaricin (1) (recorded at 400/100 MHz; δ in p.p.m., *J* in Hz)**

Position	$^{13}C^{a}$	$^{13}C^{b}$	$^{1}H^{a}$
1-NH			7.95 (s)
2	172.2	175.3	
3	86.4	88.1	
3-OH			6.20 (s)
4	77.3	79.0	3.92 (dd, 7.4, 5.3)
4-OH			5.61 (d, 5.3)
5	58.4	60.3	3.22 (ddd, 7.4, 5.3, 2.8)
6	60.5	62.1	3.31 (m)
			3.57 (ddd, 11.0, 5.3, 2.8)
6-OH			4.74 (dd, 5.3, 5.3)
7	197.8	198.4	
8	124.8	124.9	6.70 (d, 15.1)
9	141.8	145.4	7.11 (dd, 15.1, 11.3)
10	128.8	129.8	6.35 (dd, 14.7, 11.3)
11	142.7	145.1	6.74 (dd, 14.7, 10.8)
12	130.3	131.5	6.22 (dd, 14.9, 10.8)
13	140.6	142.6	5.98 (dt, 14.9, 7.1)
14	32.3	34.0	2.11 (dt, 7.1, 7.1)
15	28.1	29.8	1.37 (m)
16	30.8	32.6	1.25 (m)
17	21.9	23.6	1.25 (m)
18	13.9	14.3	0.84 (t, 6.8)

[a]Measured in DMSO-d_6.
[b]Measured in CD_3OD.

The bioactivity of **1** provides some interesting insight into structure/activity relationships. The antimicrobial activity of **1** was evaluated by a paper disc method (6 mm, Advantec Toyo Kaisha, Tokyo, Japan), as previously described.[11] The microorganisms used included *Aspergillus niger* KF 103 (ATCC 6275), *Bacillus subtilis* KB 211 (ATCC 6633), *Candida albicans* KF 1, *Escherichia coli* KB 213 (NIHJ), *Micrococcus luteus* KB 212 (ATCC 9341), *Mucor racemosus* KF 223 (IFO 4581), *Saccharomyces cerevisiae* KF 237 (ATCC 9763) and *Xanthomonas campestris* pv. *oryzae* KB 88. In essence, **1** expressed virtually no bioactivity, proving only weak activity against *B. subtilis* at 100 μg per disc. However, pramanicin exhibits potent antifungal activity against a variety of fungal pathogens, including *C. albicans* and *Cryptococcus neoformans*, as well as antibacterial activity against *B. subtilis*.[7,12] Pramanicin has also been reported to cause endothelial-dependent relaxation of rat aorta, although the activity of pramanicin A (in which the causative epoxide group was changed to a double bond) was weak.[13] While pramanicin's side-chains have two more carbons than **1**, the side-chain carbon number of TMC-260, an IL-4 signal transduction inhibitor produced by *Acremonium kiliense*,[9] was the same as that of **1**.

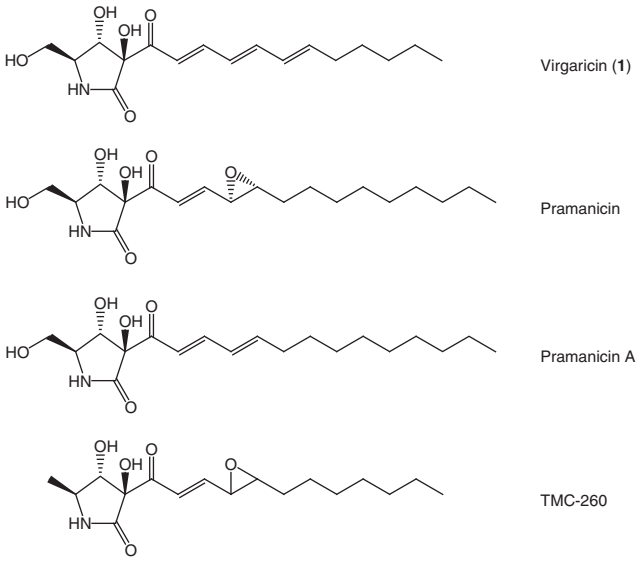

Figure 2 Structures of virgaricin (**1**) and its related compounds.

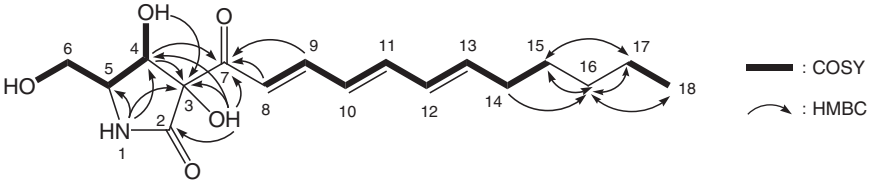

Figure 1 ^{1}H-^{1}H COSY and key HMBC correlations of virgaricin (**1**).

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