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Verticilactam, a New Macrolactam Isolated from a Microbial Metabolite Fraction Library

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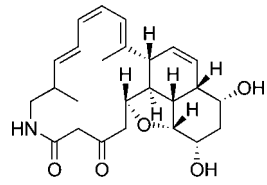
ABSTRACT



verticilactam (**1**)

Systematic isolation of microbial metabolites has been performed to construct microbial metabolite libraries or fraction libraries. A novel macrolactam, verticilactam (1), was isolated from a library of *Streptomyces spiroverticillatus* JC-8444. The structure was determined on the basis of NMR and mass spectrometric measurements. 1 had a unique 16-membered macrolactam skeleton including a *β*-keto-amide moiety.

Microorganisms possess the amazing capacity to produce various secondary metabolites[1] that have unique structures and various biological activities. Microbial metabolites have been a major source of pharmaceutical leads and therapeutic agents,[2] and there has also been a tendency to use them as potential bioprobes[3] for chemical biology studies.[4] Due to their wide range of physicochemical properties and low abundance, not all secondary metabolites have been isolated and investigated for their potentially useful activities. Recently, Bugni et al. demonstrated the advantage of a marine natural products library, constructed by an HPLC-MS fractionation protocol for rapid drug discovery.[5] We have attempted to construct a fraction library of microbial metabolites by developing a systematic separation method, using middle pressure liquid chromatography (MPLC) and HPLC, which ensure relatively high reproducibility, maintaining stable microbial cultures and productivity.

The fractions, which comprise unidentified minor components, might contain valuable compounds that have novel structures, activities, or key metabolites of a specific biosynthetic pathway. Each fraction is analyzed on an LC/MS apparatus that is attached to a photodiode array detector to reveal the UV absorption and mass spectra of each metabolite within the fractions. The results are stored in a spectral database that we have constructed to obtain useful information to identify known compounds and discover novel metabolites. We report herein the isolation and structural elucidation of a compound, designated verticilactam (**1**), which was found in a metabolite fraction library of *S. spiroverticillatus* JC-8444 through a spectral database search.

The microbial metabolite fraction library was constructed from a 14 L culture broth of a microbial strain. The same volume of acetone was added to the entire

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culture broth and then filtered to remove mycelia. The filtrate was evaporated, and the remaining aqueous phase was partitioned with EtOAc. The organic layer was concentrated generating a crude extract that was subjected to MPLC on a silica gel column with a $CHCl_3$/MeOH gradient system. The eluant was monitored by UV absorbance at 254 nm, and five fractions were collected. Each MPLC fraction was then separated by C_{18}-HPLC with a MeOH/0.05% aqueous formic acid gradient system into 48 fractions, which were to become core components of the fraction library. The aqueous layer was subjected to DIAION HP-20 column chromatography, then washed with water and subjected to the elution procedure with 20%, 50%, and 100% MeOH, and finally washed with acetone to afford four fractions for each solvent system. All isolated fractions were analyzed by LC/MS, and the spectral data of each peak within the fraction were stored in the database.[6] In the *S. spiroverticillatus* JC-8444 fraction library, the 32nd fraction of the third MPLC fraction contained a peak that had a UV absorption spectrum of λ_{max} values of 270 (sh) and 280 nm with mass spectrum of *m*/*z* values of 428 $[M + H]^+$. A search of the UV/MS database revealed that the compound was not isolated previously. It prompted us to purify by C_{18}-HPLC with acetonitrile/water (3:7) as the mobile phase, which afforded the compound **1** (1.2 mg) as a colorless amorphous solid.

The molecular formula of compound **1** was determined to be $C_{25}H_{33}NO_5$ by HRFABMS (found: *m*/*z* 428.2413 $[M + H]^+$, calcd for $C_{25}H_{34}NO_5$ 428.2437).[7] The ^{1}H NMR spectrum showed two methyl signals at δ 1.03 (d, $J = 6.4$ Hz) and 1.61 (d, $J = 0.9$ Hz) and seven olefin signals at δ 5.36 (dd, $J = 14.7$, 9.2 Hz), 5.76 (br ddd, $J = 9.6$, 1.8, 1.8 Hz), 5.88 (ddd, $J = 9.6$, 4.6, 2.3 Hz), 5.91 (dd, $J = 11.0$, 11.0 Hz), 5.98 (br dd, $J = 11.0$, 10.5 Hz), 6.09 (dd, $J = 14.7$, 11.0 Hz), and 6.17 (br d, $J = 10.5$ Hz) (Table 1). There were also three exchangeable protons at δ 3.00 (br s), 3.37, which overlapped with the H-3 signal and were verified by the integration value, and 7.08 (br d, $J = 7.8$ Hz), as revealed by the addition of D_2O in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum possessed 25 carbon signals, including 2 methyls, 4 methylenes, and 16 methines that bore 4 oxygenated and 7 olefin signals, and 3 quaternary carbons, including an olefin carbon and 2 carbonyl carbons at δ 164.2 and 206.7, which were verified by the ^{13}C DEPT and HSQC spectral data. These observations, in conjunction with the index of hydrogen deficiency of 10, suggested that **1** was a tetracyclic compound with four double bonds and two ketones. The 2D NMR spectra were measured to determine the structure. The connections between protons and carbons were determined by correlations in the HSQC spectrum. The DQF-COSY, TOCSY, and HSQC-TOCSY spectra revealed the connectivities from C-5 to C-9 and C-10 to C-16, which also

(6) Construction of a microbial metabolite fraction library and DAD-LC/MS condition was described in the Supporting Information.

(7) Verticilactam (**1**): colorless amorphous solid; $[\alpha]_{589}^{25}$ −133° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 269 (sh, 4.19), 278 (4.22); ESIMS *m*/*z* 428 $[M + H]^+$; HRFABMS found *m*/*z* 428.2413 $[M + H]^+$ calcd for $C_{25}H_{34}NO_5$ 428.2437; ^{1}H and ^{13}C NMR chemical shifts were summarized in Table 1.

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Verticilactam (**1**) in $CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C

position	δ_c	δ_H (multi, *J* in Hz)
2	164.2	-
3	48.4	3.24 (d, 16.5)
		3.36 (d, 16.5)
4	206.1	-
5	49.5	2.73 (dd, 19.7, 8.2)
		2.84 (br d, 19.7)
6	78.1	4.29 (br dd, 8.8, 8.8)
7	42.5	2.01 (m)
8	41.2	2.23 (m)
9	76.8	4.11 (dd, 8.2, 5.0)
10	67.3	4.08 eq. (m)
11	32.3	1.55 ax. (m)
		2.47 eq, (dt, 15.1, 3.7)
12	67.1	4.07 eq. (m)
13	40.3	2.45 (m)
14	129.2	5.88 (ddd, 9.6, 4.6, 2.3)
15	132.70	5.76 (ddd, 9.6, 1.8, 1.8)
16	50.70	2.80 (br ddd, 10.8, 2.3, 1.8)
17	138.80	-
18	122.99[a]	6.17 (br d, 10.5)
19	123.04[a]	5.98 (br dd, 11.0, 10.5)
20	129.00	5.91 (dd, 11.0, 11.0)
21	125.80	6.09 (dd, 14.7, 11.0)
22	137.80	5.36 (dd, 14.7, 9.2)
23	40.50	2.15 (m)
24	44.30	2.84 (m)
		3.48 (m)
25	13.20	1.61 (d, 0.9)
26	17.60	1.03 (6.4)
1-NH	-	7.08 (br d, 7.8)
10-OH	-	3.37[b]
12-OH	-	3.00 br s

[a] Interchangeable. [b] Overlapped with H-3 signal at 3.36 ppm.

connected to C-7 to form a cyclohexene as shown in Figure 1. The connectivity between C-9 and C-10 was confirmed

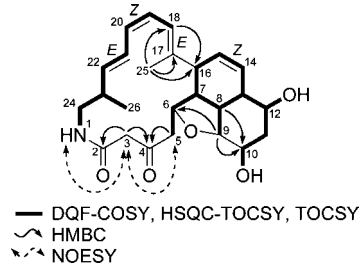


Figure 1. Structure of verticilactam (**1**) with key 2D NMR correlations.

by correlations from H-8 and H-9 to C-10 in the HMBC spectrum because they had close chemical shifts in the ^{1}H NMR spectrum and could not be distinguished from a cross peak in the DQF-COSY spectrum. The connectivities from C-18 to NH with a methyl group at the C-23 position were

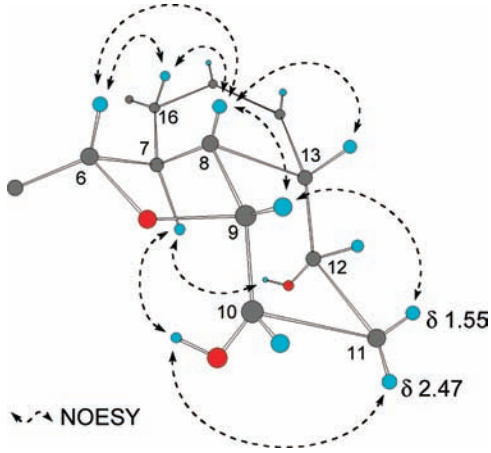


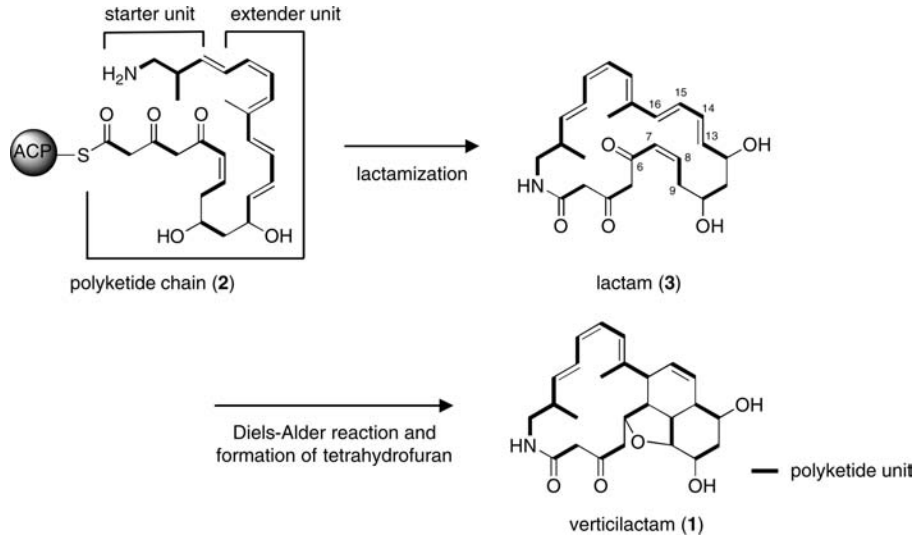
Figure 2. Key NOESY correlations in the partial 3D structure of verticilactam (**1**).

also revealed by the DQF-COSY, TOCSY, and HSQC-TOCSY spectra. In the HMBC spectrum, the H-9 signal showed a long-range correlation with C-6 to construct a tetrahydrofuran, which was also supported by ^{1}H and ^{13}C NMR chemical shifts at C-6 and C-9. A methyl signal at δ 1.61 (d, $J = 0.9$ Hz) correlated with C-16, C-17, and C-18 in the HMBC spectrum, and it was assigned to attach at C-17, which also indicated the connectivities from C-16 to C-18. This was confirmed by a long-range correlation from H-18 to C-16. The H-5 signal showed an HMBC correlation with a ketone signal at δ 206.1, which suggested that it was assigned as C-4. The H-3 signal correlated to C-4 and another carbonyl signal at δ 164.2, which revealed an assignment of the carbonyl signal as C-2 and a β-dicarbonyl moiety from C-2 to C-4. The connectivities from C-2 to C-5 were also confirmed by NOE correlations between H-3 at δ 3.36 and both H-5 signals in the phase-sensitive (ps) NOESY spectrum. An NOE correlation between NH and H-3 at δ 3.24 signals was observed, indicating an amide bond, which was supported by the relatively high-field chemical shift value of C-2. Therefore, the planar structure of **1** was determined as shown in Figure 1. The stereochemistry at double bonds at Δ^{14}, Δ^{19}, and Δ^{21} was assigned as *Z*-, *Z*-, and *E*-configurations by the coupling constant of 9.2, 11.0, and 14.7 Hz, in the ^{1}H NMR spectrum, respectively. The observation of C-25 with the typical high-field chemical shift value of δ 13.2 suggested that the double bond at Δ^{17} was assigned as an *E*-configuration.

The relative stereochemistry around the cyclohexane was assigned by correlations in the psNOESY spectrum as shown in Figure 2. The H-9 correlated with H-11 at δ 1.55, suggesting that those two protons had axial orientations on the cyclohexane. Both hydroxyl protons at C-10 and C-12 correlated with the H-7 signal, showing that the hydroxyl groups were axials, which was supported by correlations from H-10 and H-12 to both H-11 protons (not illustrated in the 3D structure in Figure 2). The hydroxyl proton at C-10 also correlated with H-11 at δ 2.47, suggesting that H-11 at δ 2.47 was an equatorial. This was supported by the lower chemical shift rather than another signal at H-11. These observations suggested that the cyclohexane had a chair conformation. The correlations between H-9 and H-8 and H-8 and H-13 suggested that H-8 was an equatorial and H-13 was an axial on the cyclohexane. The H-16 showed an NOE with H-8, indicating that the cyclohexene had a pseudochair conformation, and the H-16 was assigned as an axial on the cyclohexene. The H-8 also correlated with H-6, and the H-6 was placed on the same side as H-8. Thus, all of the relative stereochemistry around the cyclohexane was assigned as shown in Figure 2, and the structure of verticilactam (**1**) was determined to be the structure in Figure 1.

20-Membered macrolactam, vicenistatin,[8] has been reported to be biosynthesized by type I polyketide synthase

Scheme 1. Proposed Biosynthetic Pathway for Verticilactam (**1**)



(PKS), which accepts 3-methylaspartate as a starter unit. Because of the similar macrolactam structure, we expected that verticilactam (**1**) was biosynthesized by type I PKS, which utilizes 3-methylaspartate as the starter unit. The loading of the amino acid derived starter unit and polyketide chain extension by the condensation of nine malonyl-CoAs and one methylmalonyl−CoA might produce a putative post-PKS product, 24-membered lactam (**3**). We speculated that the macrolactam might be modified by potential Diels−Alder cycloaddion at Δ^7, Δ^{13}, and Δ^{15} and followed by tetrahydrofuran formation via C-9 oxidation by P450 (Scheme 1).

Compound **1** was evaluated by *in vitro* biological activities. Its antibacterial and antifungal activities against *Escherichia coli* HO-141 and *Magnaporthe oryzae* and its cytotoxicity against human promyelocytic leukemia cell HL-60 were evaluated. However, the compound had no biological effects at concentrations up to 50 μg/mL.

S. spiroverticillatus JC-8444 is also known to produce a protein phosphatase type 1 inhibitor, tautomycin,[9] which is also a member of a class of polyketides and has a 6,6-spiroacetal and a unique 2,3-dialkylmaleic anhydride moiety.[10] However, verticilactam (**1**) produced by the same microorganism has a completely different structure with an unusual β-keto-amide moiety in a 16-membered macrolactam skeleton, which, to our best knowledge, is the first example having the β-keto-amide moiety. Moreover, this result indicates that our methodology in constructing a fraction library is useful for the discovery of novel compounds.

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Supporting Information Available: Experimental procedures and NMR spectra of verticilactam. This material is available free of charge via the Internet at http://pubs.acs.org.

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