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Transformation of an Irregularly Bridged Epidithiodiketopiperazine to Trichodermamide A

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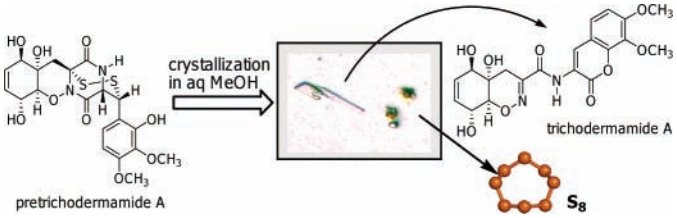
ABSTRACT



An unusually bridged epidithiodiketopiperazine, pretrichodermamide A (3), was isolated from the fungus *Trichoderma* sp. BCC 5926. During the extensive effort to crystallize 3 for X-ray crystallographic analysis, conversion of this compound to trichodermamide A with coproduction of S_8 occurred.

The epipolythiodiketopiperazines are a large class of fungal metabolites; some of them, e.g., gliotoxin, are known to possess potent antitumor activities.[1] These compounds commonly possess an −SS−, −SSS−, or −SSSS− bridge between the α-positions of the cyclic dipeptide. Gliovirin (**1**)[2] is one of the very rare "unusual" bridged analogues, wherein the disulfide links between the α- and β-positions of the amino acid residues. Trichodermamides A (**2**) and B, recently isolated from *Trichoderma virens*, strains CNL910 and CNK266, along with **1**, possess an *O*-alkyl-oxime functionality.[3−5] Although closely related, the biosynthetic relationship between **1** and **2** has remained unsolved.

As part of an ongoing research program to search for novel bioactive compounds from local fungi in Thailand, we have investigated cytotoxic constituents from a culture broth of the fungus *Trichoderma* sp. BCC 5926.[6] Chromatographic fractionation led to the isolation of a new epidithiodiketopiperazine, pretrichodermamide A (**3**), and trichodermamide A (**2**), together with two known sesquiterpenes, gliocladic acid[7] and hydroheptelidic acid,[8] and two known steroidal furans, β-viridine[9] and viridiol.[10] During the extensive effort to crystallize **3** for X-ray crystallographic

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(1) Cole, R. J.; Cox, R. H. In *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, 1981; Chapter 11, pp 569−645.
(2) Stipanovic, R. D.; Howell, C. R. *J. Antibiot.* **1982**, *35*, 1326−1329.
(3) Garo, E.; Starks, C. M.; Jensen, P. R.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **2003**, *66*, 423−426.
(4) Trichodermamide B is the 5-chloro analogue of trichodermamide A.
(5) It has been pointed out in two independent papers[3,15] that pencillazine, isolated by Lin et al., might be identical to trichodermamide A: Lin, Y.; Shao, Z.; Jiang, G.; Zhou, S.; Cai, J.; Vrijmoed, L. L. P.; Jones, E. B. G. *Tetrahedron* **2000**, *56*, 9607−9609.
(6) *Trichoderma* sp. was collected on a bamboo leaf from Khao Yai National Park, Nakhon Ratchasima Province, Northeast Thailand, by Dr. Narumol Plaingam. This fungus is deposited in the BIOTEC Culture Collection (BCC) as BCC 5926.
(7) Itoh, Y.; Takahashi, S.; Arai, M. *J. Antibiot.* **1982**, *35*, 541−542.
(8) Calhoun, L. A.; Findlay, J. A.; Miller, J. D.; Whitney, N. J. *Mycol. Res.* **1992**, *96*, 281−286.
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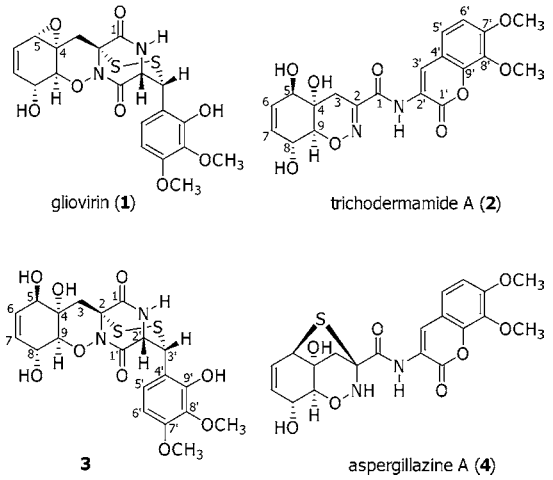
analysis, we came across a unique observation: conversion of **3** to **2** together with coproduction of S_8. We report herein our research results related to this discovery.



The fungus *Trichoderma* sp. BCC 5926 was fermented in bacto-malt extract broth (MEB) medium (5 L). EtOAc extract of the culture filtrate (6.89 g) was passed through a Sephadex LH-20 column ($MeOH/CH_2Cl_2$ = 1:1) and then subjected to repeated silica gel column chromatography (1−20% MeOH in CH_2Cl_2, step gradient elution) to obtain six pure compounds with the following order of elution: viridin (12 mg), viridiol (450 mg), gliocladic acid (30 mg), **2** (4.7 mg),[11] **3** (30 mg), and hydroheptelidic acid (630 mg).

Pretrichodermamide A (**3**) was obtained as a pale yellow solid.[12] ^1H and ^{13}C NMR and IR spectra of **3** were similar to those of gliovirin (**1**). The molecular formula of **3** was determined by HRMS and ^{13}C NMR as $C_{20}H_{22}N_2O_9S_2$. The gross structure of **3** was elucidated by 2D NMR analysis, and the absolute stereostructure of **3** was established by X-ray crystallographic analysis as depicted.[13] However, we experienced gradual degradation of **3** in our earlier effort of crystallization. Thus, upon repeated dissolving of the solid in aqueous MeOH and allowed gradual solvent evaporation, we noted the appearance of deep yellow crystals together with colorless plates. X-ray diffraction analyses revealed that the former was S_8 and that the latter was trichodermamide A (Figure 1).

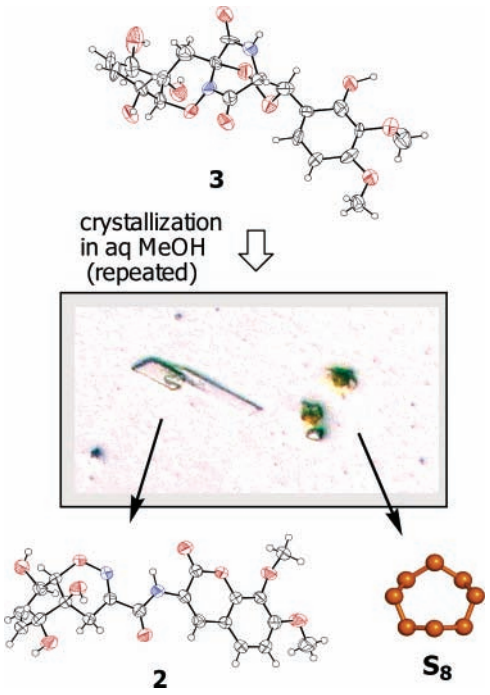


Figure 1. X-ray crystallographic structures of trichodermamide A (**2**) and S_8, and a photo of **2** and S_8 in the vial used for crystallization of **3** (taken after evaporation of the solvent). The crystal structure of pretrichodermamide A (**3**) was obtained from a separate experiment.

Two plausible pathways may be presented to account for the transformation of pretrichodermamide A (**3**) to trichodermamide A (**2**) (Scheme 1). Initial intramolecular amide−ester exchange, followed by consecutive elimination of dihydrogen disulfide (H_2S_2) would provide compound **2** (route **A**). Alternately, as shown in route **B**, β-elimination with the cleavage of H−C(2) and S−C(3) bonds would give an intermediate with proper olefinic geometry for intramolecular amide−ester exchange, followed by subsequent elimination of H_2S_2. Presumably, H_2S_2 would be readily converted to the more stable S_8 by air oxidation.[14]

Behavior of pretrichodermamide A (**3**) was examined under neutral, basic, and acidic conditions. Compound **3**

(10) Lumsden, R. D.; Ridout, C. J.; Vendemia, M. E.; Harrison, D. J.; Waters, R. M.; Walter, J. F. *Can. J. Microbiol*. **1992**, *38*, 1274−1279.

(11) Trichodermamide A (**2**): pale yellow powder; mp 249−250 °C; $[\alpha]^{26}_D$ +176° (*c* 0.22, MeOH); UV, IR, ^1H, and ^{13}C NMR spectra were identical to the literature data; HRMS (ESI-TOF) *m/z* 455.1077 (calcd for $C_{20}H_{20}N_2O_9Na$, 455.1067) $[M + Na]^+$.

(12) Pretrichodermamide A (**3**): pale yellow powder; mp 238−240 °C; $[\alpha]^{25}_D$ −206° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.60), 284 (3.59) nm; IR (KBr) ν_{max} 3920, 1691, 1607, 1506, 1466, 1333, 1258, 1093, 1019, 956, 818 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.45 (1H, s, 9′-O*H*), 9.05 (1H, d, *J* = 4.4 Hz, N*H*), 7.44 (1H, d, *J* = 8.8 Hz, H-5′), 6.55 (1H, d, *J* = 8.9 Hz, H-6′), 5.48 (1H, dt, *J* = 10.4, 2.0 Hz, H-7), 5.42 (1H, br d, *J* = 10.4 Hz, H-6), 5.26 (1H, d, *J* = 5.1 Hz, 5-O*H*), 5.22 (1H, d, *J* = 6.7 Hz, 8-O*H*), 5.09 (1H, br s, 4-O*H*), 4.49 (1H, d, *J* = 2.7 Hz, H-3′), 4.41 (1H, dd, *J* = 4.2, 3.0 Hz, H-2′), 4.23 (1H, m, H-8), 4.16 (1H, m, H-5), 3.93 (1H, d, *J* = 7.2 Hz, H-9), 3.78 (3H, s, 7′-OCH_3), 3.67 (3H, s, 8′-OCH_3), 2.09 (1H, d, *J* = 16.0 Hz, H-3a), 1.96 (1H, d, *J* = 16.0 Hz, H-3b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.1 (s, C-1), 164.8 (s, C-1′), 153.1 (s, C-7′), 148.0 (s, C-9′), 136.0 (s, C-8′), 129.9 (d, C-6), 128.9 (d, C-7), 123.1 (d, C-5′), 116.6 (s, C-4′), 103.5 (d, C-6′), 85.7 (d, C-9), 74.3 (d, C-5), 71.0 (s, C-4), 69.5 (s, C-2), 64.7 (d, C-8), 60.4 (q, 8′−OCH_3), 59.0 (d, C-2′), 55.9 (q, 7′−OCH_3), 45.0 (d, C-3′), 31.9 (t, C-3); HRMS (ESI-TOF) *m/z* 521.0659 (calcd for $C_{20}H_{22}N_2O_9S_2Na$, 521.0664) $[M + Na]^+$.

(13) Compound **3** was crystallized by slow evaporation from acetone/H_2O. Crystal data for compound (**3**) at 298(2) K: $C_{20}H_{22}N_2O_9S_2 \cdot H_2O \cdot 1/2\ C_3H_6O$, M_r = 545.58, orthorhombic, space group $P2_12_12$, *a* = 21.1106(12) Å, *b* = 9.8909(3) Å, *c* = 12.3298(6) Å, *V* = 2574.5(2) Å$_3$, D_x = 1.431 g/cm^3, *Z* = 4. F_{000} = 1164, λ(Mo Kα) = 0.71073 Å, μ = 0.27 mm^{-1}. Data collection and reduction: crystal size 0.20 × 0.15 × 0.10 mm^3, θ range 0.998−24.108°, 15 651 reflections collected, 2340 unique reflections, 1659 observed ($|F_o| > 4\sigma|F_o|$); final *R* indices, R_1 = 0.0622, wR_2 = 0.1835 for 333 parameters, GOF = 1.118. Flack parameter = −0.07(15). The coordinates were deposited to the Cambridge Crystallographic Data Centre with reference code, CCDC 605511. These data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; e-mail deposit@ccdc.cam.ac.uk).

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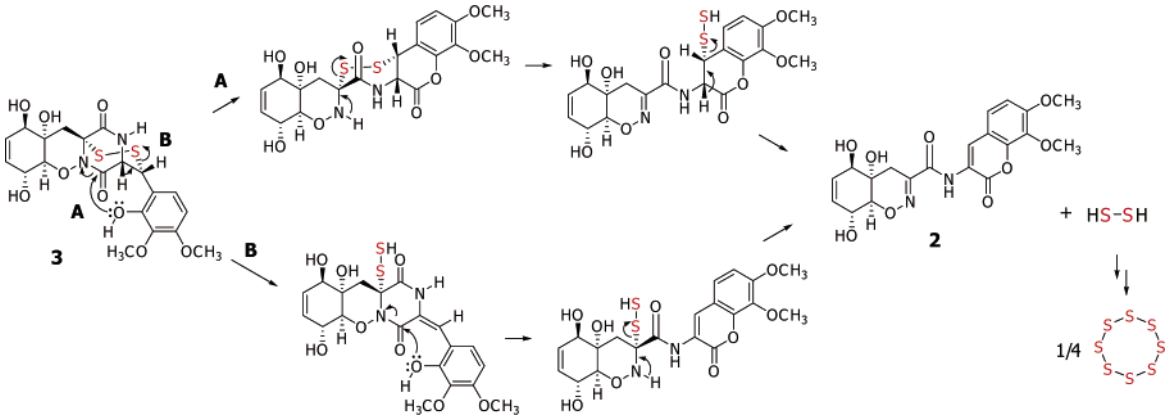
Scheme 1. Possible Transformations from **3** to **2**



gradually degraded in MeOH-d_4, giving compound **2** and unidentified products. In the presence of *p*-toluenesulfonic acid (*p*-TsOH), in MeOH-d_4, the rate of conversion of **3** to degraded compounds was similar to that in the neutral conditions. Compound **3** was stable in pyridine or pyridine-d_5/MeOH-d_4 (1:5), showing no decomposition after 3 h. In contrast, when compound **3** was treated with K_2CO_3(s) in MeOH-d_4, a rapid and clean reaction took place to give trichodermamide A as the exclusive product, as confirmed by ^{1}H NMR analysis of the filtrate after 15 min. These results indicated that the transformation of **3** to **2** was accelerated under alkaline conditions. It is consistent with the initiation steps of both pathways shown in Scheme 1: deprotonation of phenol (route **A**) or base-promoted β-elimination (route **B**).

Following these observations, we recultured the fungus BCC 5926, and the EtOAc extract was fractionated by a repeated Sephadex LH20 column and partially purified without using silica gel. Neither the crude extract nor any of the Sephadex LH20 column fractions contained compound **2**, whereas **3** and other metabolites were detected by ^{1}H NMR. Therefore, trichodermamide A (**2**) might be the isolation artifact.

Pretrichodermamide A (**3**) exhibited activity against *Mycobacterium tuberculosis* H_{37}Ra with an MIC value of 12.5 μg/mL, whereas it was noncytotoxic to Vero cells at a concentration of 50 μg/mL. Moreover, the present study clearly correlated the relationship between gliovirins and trichodermamides. It should also be mentioned that, very recently, Capon and co-workers isolated related compounds aspergillazines A−E, e.g., aspergillazine A (**4**), along with trichodermamide A, from *Aspergillus unilateralis* (MST-F8675).[15] These compounds should also be biosynthetically derived from **1** or **3**.

Acknowledgment. Financial support from the Bioresources Research Network, National Center for Genetic Engineering and Biotechnology (BIOTEC), is gratefully acknowledged. We are grateful to the BIOTEC Bioassay Laboratory and Fermentation Laboratory for biological assays and fungus fermentation. P.K. thanks the Thailand Research Fund for a research grant. S.P. thanks a scholarship from Postgraduate Education and Research in Chemistry.

Supporting Information Available: Fermentation and isolation procedures, ^{1}H and ^{13}C NMR spectra for **3**, and X-ray crystallographic data of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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