# Structure Reassignment of the Fungal Metabolite TAEMC161 as the Phytotoxin Viridiol

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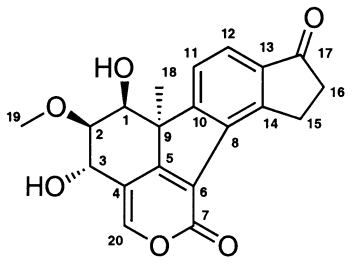
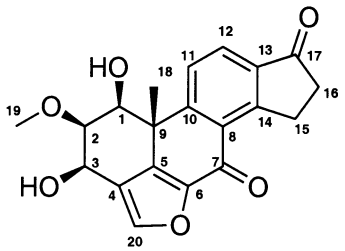
Two-dimensional NMR analyses including HMBC, NOESY, and ROESY as well as 1D NOE experiments led to a reassignment of the structure of the recently identified *Trichoderma hamatum* metabolite TAEMC161 (**1**) as the previously known viridiol (**2**). In addition, GIAO-calculated $^{13}C$ NMR chemical shifts of **1** and **2** provided strong support for the revised structure.

As part of our program toward the total synthesis of the fungal metabolite wortmannin and related natural PI-3 kinase inhibitors,[1] we became aware of the recently isolated *Trichoderma hamatum* metabolite TAEMC161 (**1**).[2] The structural similarity between **1** and the *Trichoderma viride* derived steroid viridiol (**2**) is striking;[3] however, the furanoquinone moiety of viridiol was substituted with a rather unique cyclopentapyrone in TAEMC161 (Figure 1). In contrast, a close investigation of the spectroscopic data for **1** revealed a level of equivalency with **2** that was inconsistent with this structural divergence.

Nakajima and co-workers identified a molecular formula of $C_{20}H_{18}O_6$ for **1**, which is also consistent with the chemical structure of **2**.[2] Characteristic IR absorptions for **1** were reported at 1671 (C-17, ketone) and 1707 (C-7, ester) $cm^{-1}$, in good agreement with IR frequencies reported for viridiol of 1673 (C-17, ketone) and 1712 (C-7, enone) $cm^{-1}$ ($CHCl_3$).[3a] The UV spectrum reported for **1** showed maxima at $\lambda_{max}$ (log $\epsilon$) 250 (4.19), 319 (3.86), and 399 nm (2.70), which correspond very well to UV absorption bands at $\lambda_{max}$ (log $\epsilon$) 250 (4.47) and 317 nm (4.07) described for **2**.[3a] The fragmentation pattern in the electron-impact mass spectrum (EIMS) of **1** is nearly identical to the fragmentation reported for **2**. In addition to the molecular ion at *m*/*z* 354 [$M^+$] observed for **1**, fragment ions were reported at *m*/*z* 336 ($M^+ - 18$), 308 ($M^+ - 46$), and 280 ($M^+ - 74$).[2] Similarly, in the isolation paper for **2**, the molecular ion ([$M^+$], *m*/*z* 354) was reported along with a fragmentation pattern of M-18, M-33, M-46, and M-74.[3a] The $^1H$ NMR data of metabolite **1** in two solvents, acetone-$d_6$ as well as $CDCl_3$, matched the data reported for viridiol.[3b,4] Further analysis of an authentic sample of TAEMC161 by HMQC, $^1H-^1H$ COSY, NOESY, ROESY, and 1D NOE experiments provided key correlations that were consistent with structure **2**. Specifically, irradiation of H-2 ($\delta$ 3.64) showed a 2% enhancement of both H-1 and H-3, thus indicating a *cis*-relationship between H-1, H-2, and H-3. NOESY and ROESY correlations provided further support of the stereochemical assignment (Figure 2). Through-space correlations were observed between H-1 and H-2, and H-2 and H-3. Another strong NOESY/ROESY correlation was observed between C-1 and C-3 hydroxyl protons. The methyl group (H-18) showed a correlation with H-11. H-18 did not show any correlation to H-1; however, a very weak correlation between H-18 and the C-3 hydroxyl proton was observed. The coupling constants of $J_{1,2} = 6.0$ Hz and $J_{2,3} = 4.6$ Hz are also indicative of a *cis*-relationship between the hydroxy and methoxy groups.

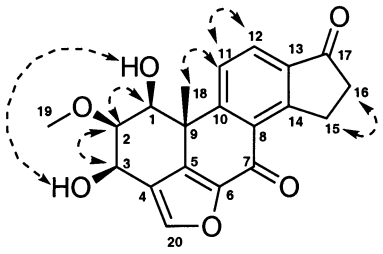



**1**




**2**

**Figure 1.** Structures originally assigned for TAEMC161 (**1**) and viridiol (**2**).




**2**

**Figure 2.** NOESY and ROESY correlations obtained for an authentic sample of **1**, consistent with structure **2**.

In strong support for structure **2**, the HMBC spectrum of TAEMC161 showed the key four-bond connectivity of the aromatic proton H-11 ($\delta$ 8.28) to the carbonyl carbon C-7 ($\delta$ 206.7) (Figure 3). Proton H-11 also correlated with C-10, C-8, C-17, C-9, and C-13, as expected, but not with C-6. The second aromatic proton, H-12 ($\delta$ 7.97), showed cross-peaks with C-10, C-13, C-14, and C-17. The furan proton H-20 ($\delta$ 7.81) correlated with C-3, C-4, C-5, C-6, and C-7. This information supports the connectivities C-11,C-10,C-8,C-7 and C-20,C-4,C-5,C-6,C-7, found only for structure

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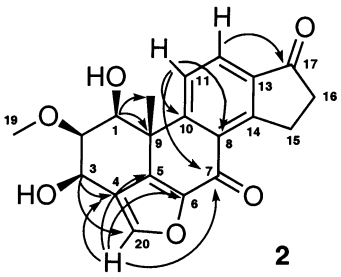


**Figure 3.** Selected HMBC correlations (H to C) for TAEMC161.

**2**. The methyl group (H-18) showed a correlation with C-9, C-1, C-5, C-10, and C-6. This indicates that C-9 is connected to C-1, C-5, and C-10. On the basis of the $^1$H NMR coupling constants and NOESY/ROESY data, C-1, C-2, and C-3 are connected. Proton H-3 showed a correlation to C-4, C-5, and C-20 (furan carbons) in addition to C-1 and C-2. These data establish the connectivity C-9,C-1,C-2,C-3,C-4,C-5. Proton H-15 is correlated with C-16, C-17, C-14, C-8, and C-13. H-16 showed correlations to C-17, the carbonyl carbon, and C-14. Overall, the HMBC data are in good agreement with structure **2**, but difficult to reconcile with structure **1**.

Recently, GIAO-based $^{13}$C NMR chemical shift calculations have emerged as a powerful tool for structure assignments of natural products.[5,6] Since the proposed structure **1** for TAEMC161 contained unique features that limited a direct shift comparison with reference compounds from the literature, we were interested in testing the scope of an ab initio computation of its $^{13}$C NMR spectrum in comparison to the alternative structure **2**. Density functional theory calculations at the B3LYP/6-311+G(2d,p) level with B3LYP/6-31G(d) optimized geometries[7] of **1** and **2** were indeed in strong support of a structural revision. The deviations of calculated versus measured $^{13}$C NMR chemical shifts are summarized in Figure 4.[8] The mean absolute error with respect to the experimentally observed chemical shifts for TAEMC161 was found to be 8.0 ppm for structure **1** versus 3.9 ppm for structure **2**. At the most significant positions C-4, C-5, C-6, C-7, and C-20, the deviations of the calculated and measured $^{13}$C NMR chemical shifts were −2.9, 24.4, −21.4, −14.5, and 7.3 ppm, respectively, for structure **1** (average = 14 ppm), versus 4.6, 2.7, 2.5, −0.1, and −1.4 ppm, respectively, for structure **2** (average = 2.2 ppm). Accordingly, the quantum-mechanical GIAO calculations of $^{13}$C NMR chemical shifts lend further independent support to this structural revision.

It is worthwhile to comment on the natural origins and the biological activities of TAEMC161 and viridiol. The *Trichoderma hamatum* fungal metabolite TAEMC161 was shown to possess activity against 5′-hydroxyaverantin dehydrogenase (HAVN).[2] Viridiol was first isolated in 1969 from a strain of the fungus *Gliocladium virens*.[3,4] This fungus was incorrectly described as *Trichoderma viride* in the early literature.[9] The structure of viridiol had been determined by UV, IR, and mass spectrometry data in addition to comparison of the $^1$H NMR data with the previously known viridin (**3**).[10] Viridiol has also been isolated from *Gliocladium deliquescens*;[3b] *Trichoderma* and *Gliocladium* strains are genetically closely related and are ubiquitous in the environment, especially in soil.[11] Biosynthetically, in *G. virens*, viridiol appears to be derived exclusively from an enzymatic and intracellular reduction of **3** based on radiolabeling studies (Figure 5).[12]

In conclusion, IR, UV and MS spectroscopy, 2D NMR, and ab initio $^{13}$C NMR shielding computations uniformly support a reassignment of the structure of the recently isolated *T. hamatum* metabolite TAEMC161 as viridiol (**2**). Our analysis also confirms that readily available ab initio computational strategies have now reached a level of sophistication that justifies their routine application in the structural analysis of complex natural products.[6,13]

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded at 300 MHz ($^1$H NMR) at room temperature using a Bruker AVANCE 300 MHz spectrometer. 2D NMR experiments were recorded on either a 500 or 600 MHz Bruker AVANCE spectrometer. Chemical shifts ($\delta$) are reported as follows: chemical shift multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constant, integration. GIAO $^{13}$C NMR chemical shift calculations were performed at the B3LYP/6-311+G(2d,p) level with B3LYP/6-31G(d) optimized geometries using G98M.[14,15]

**Spectroscopic Data for TAEMC161:** $R_f$ = 0.29 (7:3 $CHCl_3$/acetone); $^1$H NMR (300 MHz, acetone-$d_6$) $\delta$ 8.51 (d, $J$ = 8.1 Hz, 1 H), 7.93 (d, $J$ = 1 Hz, 1 H), 7.84 (d, $J$ = 8.1 Hz, 1 H), 5.06 (dd, $J$ = 5.5, 4.9 Hz, 1 H), 4.75 (d, $J$ = 6.8 Hz, 1 H), 4.52 (d, $J$ = 7.2 Hz, 1 H), 4.41 (dd, $J$ = 6.1, 4.0 Hz, 1 H), 3.84 (t, $J$ = 4.3 Hz, 1 H), 3.80−3.76 (m, 1 H), 3.71 (s, 3 H), 3.63−

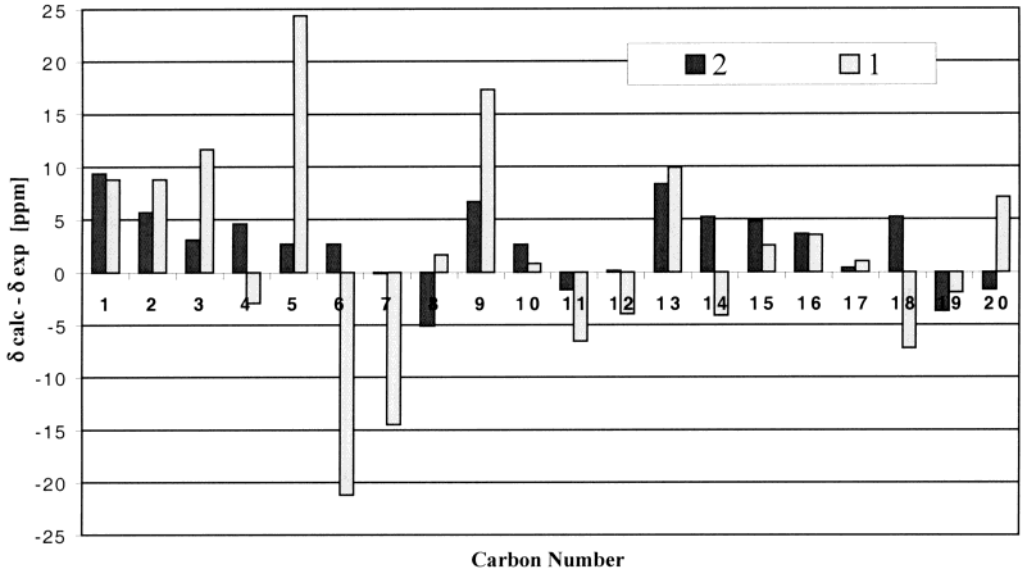


**Figure 4.** Deviations in the calculated versus measured $^{13}$C NMR chemical shifts for **1** and **2**.

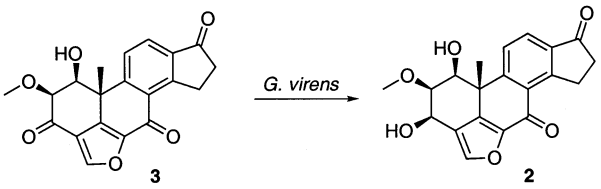


**Figure 5.** Viridin (**3**), the biosynthetic precursor of viridiol (**2**).

3.59 (m, 1 H), 2.66 (ddd, $J$ = 8.2, 4.4, 3.3 Hz, 2 H), 1.76 (s, 3 H); $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.28 (d, $J$ = 8.1 Hz, 1 H), 7.97 (d, $J$ = 8.1 Hz, 1 H), 7.81 (s, 1 H), 5.15 (d, $J$ = 4.6 Hz, 1 H), 4.34 (d, $J$ = 6.0 Hz, 1 H), 3.86−3.80 (m, 1 H), 3.74 (s, 3 H), 3.73−3.67 (m, 1 H), 3.64 (dd, $J$ = 6.2, 4.6 Hz, 1 H), 3.41 (bs, 1 H), 2.89 (brs, 1 H), 2.75 (ddd, $J$ = 8.6, 4.3, 2.7 Hz, 2 H), 1.73 (s, 3 H); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$ 206.7 (C-17), 173.5 (C-7), 158.7 (C-10), 158.1 (C-14), 145.8 (C-6), 145.6 (C-20), 142.4 (C-5), 137.0 (C-8), 129.9 (C-13), 127.4 (C-12), 127.3 (C-11), 122.1 (C-4), 81.7 (C-2), 71.8 (C-1), 61.7 (C-3), 60.8 (C-19), 42.4 (C-9), 36.5 (C-16), 30.5 (C-18), 28.5 (C-15); correlations observed in the HMBC spectrum: C-1 (H-2, H-3, H-18), C-2 (H-3, H-19), C-3 (H-1, H-20), C-4 (H-2, H-3, H-20), C-5 (H-3, H-18, H-20), C-6 (H-18, H-20), C-7 (H-11, H-20), C-8 (H-11, H-15), C-9 (H-1, H-2, H-11, H-18), C-10 (H-1, H-11, H-12, H-18), C-11 *or* C-12 (H-15), C-13 (H-11, H-12, H-15), C-14 (H-11, H-12, H-15, H-16), C-15 (H-16), C-16 (H-15), C-17 (H-11, H-12, H-15, H-16), C-18 (H-1); GIAO-based $^{13}$C NMR chemical shifts;[8] **1**: $\delta$ 207.7 (C-17), 166.8 (C-5), 159.5 (C-10), 159.0 (C-7), 154.0 (C-14), 152.9 (C-20), 139.8 (C-13), 138.6 (C-8), 124.4 (C-6), 123.5 (C-12), 120.8 (C-11), 119.2 (C-4), 90.5 (C-2), 80.6 (C-1), 73.4 (C-3), 59.7 (C-9), 58.9 (C-19), 40.0 (C-16), 31.0 (C-15), 23.3 (C-18); **2**: 207.1 (C-17), 173.4 (C-7), 163.3 (C-14), 161.3 (C-10), 148.3 (C-6), 145.1 (C-5), 144.2 (C-20), 138.2 (C-13), 131.9 (C-8), 127.6 (C-12), 126.7 (C-4), 125.7 (C-11), 87.4 (C-2), 81.2 (C-1), 64.8 (C-3), 57.1 (C-19), 49.1 (C-9), 40.1 (C-16), 35.7 (C-18), 33.3 (C-15).

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## References and Notes

(1) For representative synthetic work, see: (a) Mizutani, T.; Honzawa, S.; Tosaki, S.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4680−4682. (b) Sutherland, H. S.; Souza, F. E. S.; Rodrigo, R. G. A. *J. Org. Chem.* **2001**, *66*, 3639−3641. (c) Souza, F. E. S.; Rodrigo, R. *Chem. Commun.* **1999**, 1947−1948. (d) Carlini, R.; Higgs, K.; Older, C.; Randhawa, S.; Rodrigo, R. *J. Org. Chem.* **1997**, *62*, 2330−2331.

(2) Sakuno, E.; Yabe, K.; Hamasaki, T.; Nakajima, H. *J. Nat. Prod.* **2000**, *63*, 1677−1678.

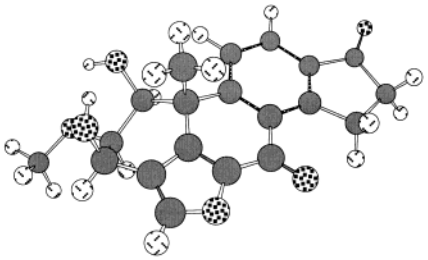
(3) (a) Moffat, J. S.; Bu'Lock, J. D.; Yuen, T. H. *J. Chem. Soc., Chem. Commun.* **1969**, 839. (b) Hanson, J. R.; O'Leary, M. A.; Wadsworth, H. J.; Yeoh, B. L. *Phytochemistry* **1988**, *27*, 387−389.

(4) Lumsden, R. D.; Ridout, C. J.; Vendemia, M. E.; Harrison, D. J.; Waters, R. M.; Walter, J. F. *Can. J. Microbiol.* **1992**, *38*, 1274−1280.

(5) Helgaker, T.; Jaszunski, M.; Ruud, K. *Chem. Rev.* **1999**, *99*, 293−352.

(6) (a) Sebag, A. B.; Forsyth, D. A.; Plante, M. A. *J. Org. Chem.* **2001**, *66*, 7967−7973. (b) Barone, G.; Duca, D.; Silvestri, A.; Gomez-Paloma, L.; Riccio, R.; Bifulco, G. *Chem. Eur. J.* **2002**, *8*, 3240−3245. (c) Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Chem. Eur. J.* **2002**, *8*, 3233−3239. (d) Betz, J.; Bauer, W. *J. Am. Chem. Soc.* **2002**, *124*, 8699−8706. (e) Della Monica, C.; Randazzo, A.; Bifulco, G.; Cimino, P.; Aquino, M.; Izzo, I.; De Riccardis, F.; Gomez-Paloma, L. *Tetrahedron Lett.* **2002**, *43*, 5707−5710. (f) Tilley, L. J.; Prevoir, S. J.; Forsyth, D. A. *J. Chem. Educ.* **2002**, *79*, 593−600.

(7) Ball-and-stick model of the B3LYP/6-31G(d) minimized structure of **2**:



(8) TMS has recently been found to be inappropriate as a computational reference for DFT calculations of the chemical shifts of ring carbons in aromatic-like systems (Schuler, R. H.; Albarran, G.; Zajicek, J.; George, M. V.; Fessenden, R. W.; Carmichael, I. *J. Phys. Chem. A* **2002**, *106*, 12178−12183). Therefore, all $sp^2$-hybridized carbons were referenced to benzene, whereas all $sp^3$-hybridized carbons were referenced to TMS as the computational standard.

(9) The amino acid sequences of the endochitinase of the fungal species *Trichoderma viride* and *Gliocladium virens* were found to be highly homologous, see: Nakahara, K.; Yoshida, K.; Ito, T.; Suzaki, K.; Kudo, A. *Arch. Phytopath. Plant Prot.* **2001**, *33*, 519−527.

(10) Grove, J. F.; McCloskey, P.; Moffatt, J. S. *J. Chem. Soc. (C)* **1966**, 743−747.

(11) Ghisalberti, E. L. *Curr. Med. Chem.* **2002**, *1*, 343−374.

(12) Jones, R. W.; Hancock, J. G. *Can. J. Microbiol.* **1987**, *33*, 963−966.

(13) (a) Kondru, R. K.; Wipf, P.; Beratan, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 2204−2205. (b) Ribe, S.; Kondru, R. K.; Beratan, D. N.; Wipf, P. *J. Am. Chem. Soc.* **2000**, *122*, 4608−4617. (c) Perry, T. L.; Dickerson, A.; Khan, A. A.; Kondru, R. K.; Beratan, D. N.; Wipf, P.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2001**, *57*, 1483−1487. (d) Specht, K. M.; Nam, J.; Ho, D. M.; Berova, N.; Kondru, R. K.; Beratan, D. N.; Wipf, P.; Pascal, R. A.; Kahne, D. *J. Am. Chem. Soc.* **2001**, *123*, 8961−8966. (e) Beratan, D. N.; Kondru, R. K.; Wipf, P. In *Chirality: Physical Chemistry*; Hicks, J. M., Ed.; American Chemical Society: Washington, DC, 2002; Vol. 810, pp 104−118.

(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Rega, N.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.11.4; Gaussian, Inc.: Pittsburgh, PA, 2002.

(15) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys.* **1996**, *104*, 5497−5509.

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