Vertinoid Polyketides from the Saltwater Culture of the Fungus *Trichoderma longibrachiatum* Separated from a *Haliclona* Marine Sponge†

Sam Sperry,‡ Gary J. Samuels,§ and Phillip Crews*,‡

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California, 95064, and Systematic Botany and Mycology Laboratory, U.S. Department of Agriculture-ARS, Beltsville, Maryland, 20705-2305

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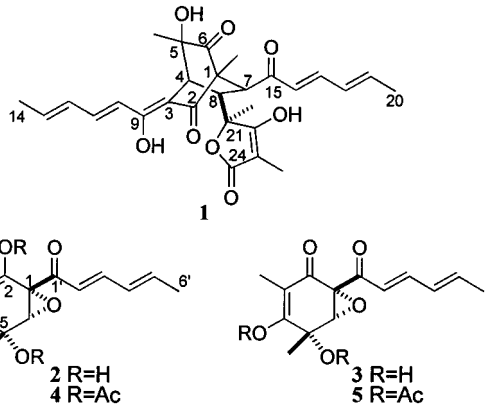
It is somewhat amazing that metabolites discovered to date from the cultures of marine-derived fungi are either parallel or identical to those of terrestrial fungi.[1] This may be analogous to the less pronounced phenomenon of parallel metabolism observed for the metabolites of some marine and terrestrial macroorganisms.[2] We are attempting to gain insights into the biosynthetic breadth possible for marine-derived fungi by focusing on tropical invertebrates, especially sponges, in order to develop a repository of saltwater fungal cultures. Work is then prioritized by using a combination of bioassay (antimicrobial, brine shrimp toxicity, and cytotoxicity) and molecular ion (electrospray ionization mass spectrometry [ESIMS]) profiling. Such screening methods led us to discover distinctive compounds with interesting properties including a diketopiperazine dimer, asperazine,[3] a series of chlorinated terpenoids, the chloriolins,[4] and a family of chlorinated polyketides, the chlorcarolides.[5] Other slightly unusual polyketides have also been uncovered via this approach including the pitholides,[6] the nectriapyrones,[7] and secocurvularin.[8] Recently, we began a study of the saltwater culture of a *Trichoderma longibrachiatum* procured from a *Haliclona* marine sponge. Species of the genus *Trichoderma* (Persoon 1821)[9] are of current interest as sources of unique metabolites. In fact, two recent independent studies simultaneously reported the same C_{28} bisvertinoid polyketide,[10] **1**, named as bislongiquinolide from *T. longibrachiatum*[11] or tricho-

Chart 1



tetronine from a *Trichoderma* sp.[12] Herein we disclose that the saltwater culture of a sponge-derived *T. longibrachiatum* also produces **1** accompanied by a new C14 vertinoid pigment, **2**, which we have named epoxysorbicillinol on the basis of the nomenclature introduced by Cram in 1948.[13]

Results and Discussion

A saltwater culture of *T. longibrachiatum* was obtained from *Haliclona* sp. during an expedition to Sulawesi, Indonesia. Initially it was grown on a solid marine media (cellulose-powder agar),[4] and after successive subculturing, an agar plug containing mycelium was used to inoculate a liquid malt extract medium. Standard growth and scale-up procedures were used to obtain 8 L of mycelium-containing broth. After separation of mycelium and broth by filtration, the broth was extracted with EtOAc. The two pure vertinoids, **1** and **2**, were obtained from the crude oil by a process beginning with solvent partitioning, fractionation by size-exclusion column chromatography, and finally HPLC purification (Chart 1).

The structure elucidation of the smaller mass compound, **2**, commenced when the molecular formula of $C_{14}H_{16}O_5$ was established by an LRESIMS *m/z* 263 $[M - H]^-$ in negative-ion mode, and a *m/z* of 265 $[M + H]^+$ in positive-ion mode. This result was eventually validated by an HRFABMS *m/z* 265.1076 $[M + H]^+$ (calcd for $C_{14}H_{17}O_5$, 265.1076). The NMR data, initially obtained in CD_3OD, proved to be puzzling, as only 10 carbon signals were visible when the spectrum was acquired under normal conditions at 125.7 MHz. Six of these resonances, (δ 196.6 s, 147.4 d, 144.5 d, 131.7 d, 124.6 d, 19.1 q), indicative of a sorbyl side chain (C1′−C6′), were further supported by the ^{1}H NMR data consisting of a dd signal at δ 7.32 (J = 15.5, 10 Hz, H3′), a complex multiplet from δ 6.29−6.44 (H2′, H4′, H5′), and a methyl doublet at δ 1.89 (J = 6 Hz, $H_3$6′). Additionally, representative MS/MS daughter ions appearing at *m/z* 67 $[C_5H_7]^+$ and 95 $[C_6H_7O]^+$ were also supportive of the C1′−C6′ unit, which accounted for three of the seven unsaturations. Another ^{13}C NMR spectrum obtained with a delay time of 10 s revealed two additional oxycarbon resonances (δ 70.6 s, C5; and 63.9 s, C1). One more

* To whom correspondence should be addressed: Tel: (831) 459-2603. Fax: (831) 459-2935. E-mail: phil@chemistry.ucsc.edu.

† Dedicated with respect to the memory of Dr. Dean E. McHenry, Founding Chancellor of UCSC.

‡ University of California, Santa Cruz.

§ U.S. Department of Agriculture−ARS.

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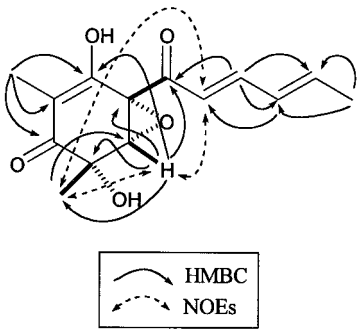


Figure 1. Important 2D NMR correlations for epoxysorbicillinol (**2**).

carbon signal (δ 173, C2) was detected indirectly via its HMBC correlations to H6 and Me8. The $^{13}C/^{1}H$ NMR signals for Me8 (δ 8.0 q/1.68 s) implied that it was attached to the 2-position of a 1,3-enolized diketo system, but one low-field sp^2 carbon was still missing.

The ^{1}H NMR spectra of **2**, acquired in several solvents, indicated that DMSO-d_6 would be most suitable for additional experiments as both enolic (δ 10.85, 2-OH) and normal alcohol (δ 6.18, 5-OH) protons were visible. The ^{13}C NMR spectrum obtained in DMSO-d_6 (15 s delay) exhibited 14 signals, with the evasive resonance for C4 appearing at δ 187.7. The HMBC experiment was redone in DMSO-d_6 and these data, summarized in Figure 1, along with biogenetic considerations (vide infra) guided the construction of a six-membered enone ring with sorbyl unit and methyls arranged as shown. The three additional oxygenated carbons (C1, C5, C6) were assigned to epoxide and alcohol residues, thus accounting for the last unsaturation unit. The 1,6-epoxy was consistent with the experimental ^{13}C values, and the alternative structure of C1−OH and 5,6-epoxy groups is ruled out because the ^{13}C NMR shift at C1 is predicted to be δ 90. In addition, H-bond stabilization dictates that the preferred tautomer is **2** and not **3**.

To further verify the arrangement of atoms in **2**, a small sample was subjected to overnight acetylation. Analytical LCESIMS of the product mixture revealed two diacetate isomers (*m/z* 349), with different retention times, that were ultimately separated by semipreparative reversed-phase HPLC. The ^{1}H and ^{13}C NMR data suggested that tautomers **2** and **3** both underwent respective conversions to **4** and **5,** probably an effect of base-shifted equilibrium. Only very small quantities (<1 mg) of each acetate derivative were obtained which limited product analysis; however, comparisons to calculated model chemical shifts[14] and the expected shift displacement of H5 from δ 3.59 to 3.96 support the corresponding structures shown for **4** and **5**. A ratio of approximately 3:1 (**4**:**5**) could be estimated by measuring pertinent ^{1}H NMR peak integrations in the product mixture.

With the gross structure of **2** defined, our attention was next shifted to deciphering its relative and absolute stereochemistry. The NOESY and difference NOE spectra (Figure 1) were useful. Selective irradiation of H6 gave substantial enhancement of Me7 and moderate enhancement of H2′. Complementary information obtained by irradiation of Me7 indicated a syn orientation for the

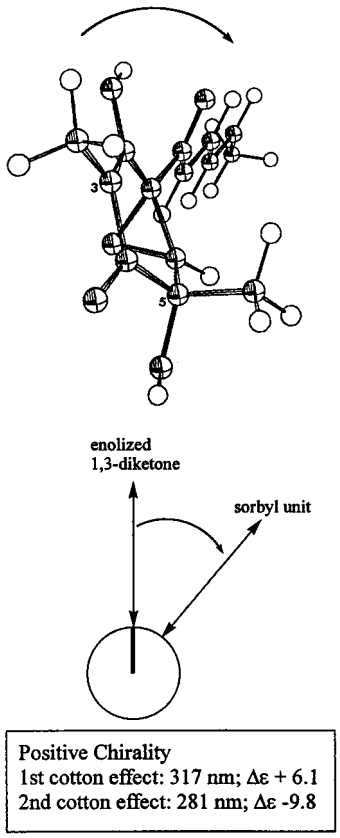


Figure 2. Molecular-model-generated ORTEP representation of epoxysorbicillinol (**2**) and CD exciton coupling analysis.

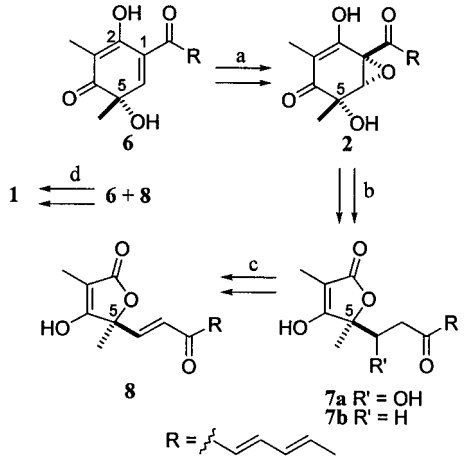
three residues. Additional stereochemical insight was provided by a CD spectrum, as the two chromophores in **2** exhibited considerable exciton interaction.[15] The Davydov splitting pattern (λ_{max} 287 nm; $\Delta\lambda = 36$ nm) consisted of two cotton effects, first, positive, and second, negative, signifying positive exciton chirality (right-handed twist) as illustrated in Figure 2 and supporting a 1*S*,5*S*,6*S* stereochemistry.

The structure elucidation of the bisvertinoid **1** proceeded in a smooth fashion with none of the NMR difficulties encountered for that of **2**. Its formula, $C_{28}H_{32}O_8$, was established by the HRFABMS *m/z* 519.1996 $[M + Na]^+$ (Δ − 0.2 mmu of calcd). Also evident from the NMR data was the presence of two sorbyl units and a tetronic acid moiety. The 2D NMR data was used to propose the gross structure, **1**, based on these substructures plus the additional moieties consisting of two methyls and a $C_8H_4O_3$ bicyclo[2.2.0]octane ring. Shortly after this was done, we became aware of two publications reporting on **1** that reported properties that were consistent with our data.[11,12] The structure and relative stereochemistry at the six chiral carbons of **1**, published by Ayer,[11] was justified by NMR data including NOESY correlations, chemical shift values, and coupling constants. Surprisingly, no comments were made about the absolute configuration of this molecule. The chiroptical properties agree well for all three samples, and the few discrepancies in the NMR data are primarily in the region of the two 1,3-dicarbonyl chromophores. This implies that there

(14) (a) A discussion of the results appears in the Ph.D. dissertation of Sperry. Sperry, S. University of California, Santa Cruz, CA, 1998; pp 199−200. (b) The calculation package used was ACD/HNMR DB v 2.51, 1997, Advanced Chemistry Development, Inc., Toronto, Canada, M5H 2L3.

(15) (a) Crews, P.; Rodríguez, J.; Jaspars, M. *Organic Structure Analysis*; Oxford University Press: New York, 1998; pp 378−379. (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy−Exciton Coupling in Organic Chemistry*; University Science Books: Mill Valley, CA, 1983.

Scheme 1. Biosynthetic Analysis. (a) Epoxidation; (b) Lactonization, Ring Cleavage, and Reduction; (c) Dehydration; (d) Diels−Alder Cyclization of 6 and 8



are varying tautomeric forms present for the different samples. The structure of **1** drawn by Satake[12] showed the two β-dicarbonyl substructures as noncommitted enols. However, the ^{13}C shifts suggest atoms C22 (δ 194.9), C23 (δ 90.3), and C24 (δ 182.2) are in the other possible tautomeric arrangement as opposed to that shown here for **1**.[16] Also, the substructure (C2, C3, and C9) of Satake's sample appears to be in rapid equilibrium between the two possible tautomers (δ 184.6, 110.5, and 181.0 as opposed to data shown for **1** in the Experimental Section). A lowered pH could explain the rapidly interconverting tautomers in the NMR spectra of that sample.[17] As a final point, some of the stereochemical conclusions reported by Satake appear to be equivocal.[12] The NOEs used to set the relative configuration at C21 may not be reliable since the tetronic acid is planar and the C8−C21 bond has free rotation. Additionally, the absolute stereoassignment (of 21*R*,5*S*) based on CD exciton coupling may not be valid because the limitations that must be satisfied to apply this method to a triple chromophore system are not present in **1**.[15b]

The vertinoid biosynthesis pathway shown in Scheme 1 is an extension of Dreiding's previous presentation,[10] in part, based on analogies to the biosynthesis of penicillic acid from orsellinic acid. Key concepts include the idea that all structures belonging to the vertinoid structure class are composed of 2,4-dimethyldodecyl units and originate from sorbicillin (see Figure S1, Supporting Information), a hexaketide which has been isolated from several fungi.[18] The enolized dicarbonyl, **6**, is a suspected precursor in the formation of several bis adducts including sorbiquinol,[19] trichodimerol,[18a] and **1**.[11,12] Further evidence of the intermediate **6** is suggested by the new epoxysorbicillinol (**2**). The latter has functionality that can undergo lactonization, ring cleavage, and reduction, to afford 5-hydroxyvertinolide (**7a**).[11] Dehydration of **7a** would produce the proposed dienophile (**8**), as suggested by Satake, to cyclize with **6** in the formation of **1**.[12] An implication of the pathway shown in Scheme 1 is that stereochemistry, of the tertiary oxycarbon arising from C5 in **6**, is conserved with each step. Our finding of 5*S* stereochemistry in **2** is consistent with this approach, originally postulated[10] when the 5*S* configuration was unequivocally determined for (−)-vertinolide (**7b**) by total synthesis.[16b,20] Finally, this suggests the 5*S*,21*S* stereochemical designations shown here for **1**.

An earlier publication disclosed the structure of trichoharzin from a sponge-derived *T. harzianum*,[21] making epoxysorbicillinol (**2**) the second novel metabolite to be discovered from a sponge-derived *Trichoderma*. The marine macroorganism source of these cultures is not surprising, as *Trichoderma* has been isolated from numerous aqueous saline environments including salt marshes, swamps, mangroves, estuaries, and seawater (eulittoral and oceanic zones).[9,22] Terrestrial sources of *T. longibrachiatum* are highly diverse and include soil, wood, tree roots, herbaceous tissue, and humans.[23] To the best of our knowledge, epoxysorbicillinol (**2**) is the fourteenth constituent of the vertinoid class, a polyketide group predominantly isolated from *T. longibrachiatum* and *Verticillium intertextum* (see Figure S1, Supporting Information). The most distinguishing characteristic of **2** is the epoxide, which also substantiates Dreiding's hypothesis that an epoxidase was involved in the vertinoid biosynthetic pathway. The chemically diverse profile exhibited by *Trichoderma* spp.[24] includes several epoxides, highlighted by heptelidic acid,[25] the isonitrins,[26] and the tricothecenes.[27] However, **2** represents the first vertinoid polyketide possessing epoxide functionality.

Experimental Section

The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.7 MHz, respectively. Atom connectivities were made by using HMQC[28,29] data to determine one-bond H−C connectivities, HMBC[29] to determine two- and three-bond H−C connectivities, and COSY data to determine three-bond H−H connectivities. High performance liquid chromatography (HPLC) was performed on columns of 10-μm ODS using a binary gradient HPLC system with a variable-wavelength UV monitor. MS data were obtained using a triple-quadrupole ESI mass spectrometer.

Collection and Identification. A small piece of *Haliclona* sp. sponge (coll. no. 96556) was excised and placed in a sterile Whirlpack bag underwater (ca. 60 ft, coral wall). At the surface,

(16) For models of this tautomer trapped as the Me-enolate see: (a) Trifonov, L. S.; Bieri, J. H.; Prewo, R.; Dreiding, A. S.; Rast, D. M.; Hoesch, L. *Tetrahedron* **1982**, *38*, 397. (b) Takaiwa, A.; Yamashita, K. *Agric. Biol. Chem.* **1983**, *47*, 429.

(17) In ref 12, isolation techniques involved acid workup and $CDCl_3$ was used as the NMR solvent. Either of these may have contributed to a pH decrease.

(18) Sorbicillin comes from the following fungi. (a) *Trichoderma longibrachiatum*: Andrade, R.; Ayer, W. A.; Mebe, P. P. *Can. J. Chem.* **1992**, *70*, 2526. (b) *Verticillium intertextum*: Trifonov, L. S.; Bieri, J. H.; Prewo, R.; Dreiding, A. S.; Hoesch, L.; Rast, D. M. *Tetrahedron* **1983**, *39*, 4243. (c) *Penicillium chrysogenum* (*notatum*): ref 13 and Arima, K.; Nakumura, H.; Komagata, K. *J. Agric. Chem. Soc. Jpn.* **1953**, *27*, 345.

(19) Andrade, R.; Ayer, W. A.; Trifonov, L. S. *Can. J. Chem.* **1996**, *74*, 371.

(20) (a) Wrobel, J. E.; Ganem, B. *J. Org. Chem.* **1983**, *48*, 3767. (b) Desmaele, D. *Tetrahedron* **1992**, *48*, 2925.

(21) Kobayashi, M.; Uehara, H.; Matsunami, K.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 7925.

(22) Johnson, T. W., Jr.; Sparrow, F. K. *Fungi in Oceans and Estuaries*; J. Cramer: 3301 Lehre, Germany, 1970.

(23) (a) Figures 2 and 4 in: Bissett, J. *Can. J. Bot.* **1984**, *62*, 924−931. (b) Samuels, G. J.; Petrini, O.; Kuhls, K.; Lieckfeldt, E.; Kubicek. C. P. *Stud. Mycol.* **1998**, in press.

(24) Over 100 entries in: *Dictionary of Natural Products on CD-ROM: Version 6.2*; Chapman and Hall: London, 1998.

(25) Itoh, Y.; Kodama, K.; Furuya, K.; Takahashi, S.; Haneishi, T.; Takiguchi, Y.; Arai, M. *J. Antibiot.* **1980**, *33*, 468.

(26) Fujiwara, A.; Okuda, T.; Masuda, S.; Shiomi, Y.; Miyamoto, C.; Sekine, Y.; Tazoe, M.; Fujiwara, M. *Agric. Biol. Chem.* **1982**, *46*, 1803, 1811.

(27) Cole, R. J.; Cox, R. H. *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, 1981; pp 152−263.

(28) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

(29) Bax, A.; Sparks, S. W.; Torchin, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 7926.

sterile conditions were used to transfer the sponge piece to a solid marine medium (cellulose-powder agar) previously described.[4] After transport back to our home laboratory, subcultures revealed the release of a yellow pigment into the solid agar medium. A plug of the agar containing mycelium (strain no. 961015) was transferred to a culture broth (malt extract) and subjected to standard scale-up procedures.[4] It yielded 8 L of broth. The fungus was identified as *Trichoderma longibrachiatum* (Rifai, 1969) as follows. Colonies grown on potato dextrose agar (Difco) in darkness for 70 h have a radius of 70 mm at 30 °C and 45 mm at 40 °C. An intense yellow pigment often spreads through the agar. Conidia in colonies grown on cornmeal dextrose agar (CMD, Difco cornmeal agar + 2% dextrose) arise along the length of aerial hyphae or in continuous, confluent, pulvinate aggregates. Phialides, arising from (1.2−)2.2−3.2(−4.5) μm-wide hyphae, cylindrical or slightly swollen in the middle, (2.2−)5.5−9.2(−16.0) μm long, (1.0−)2.2−3.2(−4.2) μm at the widest, and (1.0−)1.5−2.2(−4.5) μm at the base, form near the tip of the conidiophores, which tend to be solitary, often subtended by a single cell from which arises, laterally, just below the septum, a short, spurlike phialide. Conidia are oblong to ellipsoidal, (2.5−)3.5−4.7(−7.7) × (2.0−)2.5−3.0(−4.0) μm, and smooth. Chlamydospores are generally abundant on CMD, terminal, and globose to subglobose, or intercalary, and then conforming to the shape of the cell in which they form (3.7−)-6.0−9.7(−18.2) μm. Additional descriptions and illustrations can be found in the literature.[23]

Extraction and Isolation. The mycelium and broth were separated by filtration and each examined independently. The broth was extracted with EtOAc for 15 h on a continuous liquid−liquid extractor, after which the organic portion was concentrated by rotoevaporation, yielding a red-orange oil (1.4 g). Concurrently, the mycelium was soaked in MeOH to extract organics. After ESIMS data were obtained for both the broth and mycelium, it was decided that work would only proceed with the broth-derived EtOAc extract. The extract was partitioned between 90% aqueous MeOH and hexanes (0.14 g), upon which the percent water was increased to produce a 50% aqueous MeOH solution for additional partitioning with CH_2Cl_2 (1.02 g). Half of the CH_2Cl_2 portion (0.5 g) was subjected to size-exclusion chromatography over Sephadex LH-20 (3:2 CH_2Cl_2/MeOH), yielding eight fractions. The third Sephadex fraction contained **2**, and it was subsequently purified by reversed-phase HPLC (75% aqueous MeOH/0.1% TFA). The sixth Sephadex fraction contained **1**, and it was similarly purified (60% aq MeOH/0.1% TFA).

Bislongiquinolide (Trichotetronine) (1). Yellow amorphous powder (6.5 mg isolated). $[\alpha]_D$: + 134° (*c* 0.07, CH_3OH). UV (CH_3OH): λ_{max} (ϵ) 370 (23 500), 292 (24 100), 262 (23 500). CD (CH_3OH): λ, nm ($\Delta\epsilon$, dm^3 mol^{-1} cm^{-1}) 360 (+ 14.2), 320 (−23.4), 281 (+9.2), 235 (−10.8). IR (neat) ν 3330, 1674, 1472, 1442, 1382, 1201, 1141, 848, 800, 724 cm^{-1}. LRESIMS: positive ion *m*/*z* 519 [M + Na]$^+$, negative ion 495 [M − H]$^-$. LRFABMS *m*/*z* 519 [M + Na]$^+$. HRFABMS *m*/*z* 519.1996 (Δ − 0.2 mmu of calcd). ^{13}C NMR* (CD_3OD, 125.7 MHz): δ 210.2 (C6), 202.3 (C15), 197.6 (C2), 178.7 (C22), 176.6 (C24), 169.8 (C9), 147.9 (C17), 145.2 (C19), 144.0 (C11), 140.9 (C13), 132.4 (C18), 131.7 (C12), 128.5 (C16), 119.6 (C10), 110.1 (C3), 98.2 (C23), 84.5 (C21), 75.9 (C5), 63.6 (C1), 53.0 (C7), 44.0 (C8), 43.5 (C4), 24.3 (CH_3-C1), 23.6 (CH_3C21), 19.2 (CH_3C20), 19.0 (CH_3C14), 11.4 (CH_3-C5), 6.6 (CH_3C23). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.30 (dd, *J* = 15, 11 Hz, H11), 7.19 (dd, *J* = 15.5, 11 Hz, H17), 6.21−6.37 (m, H10, H12, H13, H16, H18, H19), 3.37[#] (br d, *J* = 1.5 Hz, H4), 3.30[#] (br d, *J* = 6 Hz, H8) 3.21 (d, *J* = 7 Hz, H7), 1.89 (d, *J* = 6.5 Hz, $H_3$14, $H_3$20), 1.49 (s, MeC23), 1.42 (s, MeC21), 1.17 (s, MeC5), 0.99 (s, MeC21). *Assignments are based on 2D data (HMBC, HMQC, COSY, APT) and comparison to models.[10,16a,18,19] [#]These values were determined in $CDCl_3$, due to CD_2HOD occlusion.

Epoxysorbicillinol (2). Yellow amorphous powder (17.5 mg isolated). $[\alpha]_D$: + 75° (*c* 0.15, CH_3OH). UV (CH_3OH): λ_{max} (ϵ) 287 nm (16 900). CD (CH_3OH) λ, nm ($\Delta\epsilon$, dm^3 mol^{-1} cm^{-1}) 317 (+ 6.1), 281 (−9.8). IR (neat): ν 3425, 1678, 1440, 1389, 1204, 1138, 843, 800, 721 cm^{-1}. LRESIMS: positive ion *m*/*z* 265 [M + H]$^+$. MS/MS: daughter ion 67 (100) [C_5H_7]$^+$, 95 (45) [C_5H_7O]$^+$, negative ion 263 [M − H]$^-$. LRFABMS: *m*/*z* 265 [M + H]$^+$. HRFABMS *m*/*z* 265.1076 (Δ − 0.0 mmu of calcd). ^{13}C NMR (DMSO-d_6, 40 μL-nanoprobe, delay time = 15 s/CD_3OD; 5 mm probe, delay time = 10 s; 125.7 MHz): δ 192.3/194.6 (C1′), 187.7/− (C4), 173.4/173* (C2), 145.0/147.4 (C3′), 143.3/144.5 (C5′), 130.3/131.7 (C4′), 124.1/124.6 (C2′), 105.6/108.0 (C3), 68.8/70.6 (C5), 62.7/63.9 (C1), 61.3/62.9 (C6), 26.0/26.2 (C7), 18.9/19.1 (C6′), 8.0/8.0 (C8). ^{1}H NMR (DMSO-d_6/CD_3OD, 500 MHz): δ 10.85/− (s, 2 OH), 7.17/7.32 (dd, *J* = 15.5, 10 Hz, H3′), 6.29−6.44/6.29−6.42 (m, H2′, H4′, H5′), 6.31 (NOE detection in DMSO-d_6, d, *J* = 16 Hz, H2′), 6.18/− (br s, 5 OH), 3.68/3.59 (s, H6), 1.85/1.89 (d, *J* = 6 Hz, Me6′), 1.57/1.68 (s, Me8), 1.44/1.49 (s, Me7). *This shift was implied inversely via HMBC cross-peaks.

Preparation of Diacetates 4 and 5. Approximately 3.5 mg of pure **2** was dissolved in dry Ac_2O−pyr (1:1). A catalytic amount of DMAP was added, and the mixture was stirred overnight at room temperature. After it was determined by ESIMS that a diacetate was formed (*m*/*z* 349 [M + H]$^+$), the reaction mixture was dried by rotoevaporation. Reversed-phase LCESIMS in 60% aqueous MeOH indicated that a mixture of diacetates was present, and identical conditions were used to achieve final HPLC separation of **4** and **5**.

2,5-Di-*O*-acetyl-epoxysorbicillinol (4). Yellow amorphous powder (<1 mg recovered). $[\alpha]_D$ could not be accurately measured. UV (CH_3OH): λ_{max} (ϵ) 262 (2000), 302 (2100). IR (neat): ν 1678, 1508, 1437, 1372, 1202, 1132, 844, 798, 720 cm^{-1}. LRESIMS: positive ion *m*/*z* 349 [M + H]$^+$. ^{13}C NMR (CD_3OD, 125.7 MHz): δ 148.8 (C3′), 145.3 (C5′), 131.8 (C4′), 120.9 (C2′), 60.6 (C6), 23.9 (C7), 21.1 (CH_3-acyl), 20.7 (CH_3-acyl), 19.2 (C6′), 10.0 (C8), no quaternary carbons appeared. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.39 (dd, *J* = 15.5, 10.5 Hz, H3′), 6.29−6.42 (m, H2′, H4′, H5′), 3.96 (s, H6), 2.19 (s, H_3-acyl), 2.11 (s, H_3-acyl), 1.89 (d, *J* = 6.5 Hz, H6′), 1.75 (s, Me8), 1.51 (s, Me7).

4,5-Di-*O*-acetyl-epoxysorbicillinol (5). Yellow amorphous powder (<1 mg recovered). $[\alpha]_D$ could not be accurately measured. UV (CH_3OH): λ_{max} (ϵ) 244 (2200), 288 (3600). IR (neat): 1678, 1437, 1372, 1202, 1132, 838, 778, 726 cm^{-1}. LRESIMS: positive ion *m*/*z* 349 [M + H]$^+$. ^{13}C NMR (CD_3OD, 125.7 MHz): δ 148.3 (C3′), 145.3 (C5′), 131.8 (C4′), 124.3 (C2′), 60.5 (C6), 22.8 (C7), 21.6 (CH_3-acyl), 20.6, (CH_3-acyl), 19.3 (C6′), 10.1 (C8), no quaternary carbons appeared. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.34 (dd, *J* = 15, 10 Hz, H3′), 6.29−6.43 (m, H2′, H4′, H5′), 4.32 (s, H6), 2.29 (s, H_3-acyl), 2.07 (s, H_3-acyl), 1.89 (d, *J* = 6.5 Hz, H6′), 1.78 (s, Me8), 1.64 (s, Me7).

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Supporting Information Available: Figures S1, S2, and S3 (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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