Dimerization of Resveratrol by the Grapevine Pathogen *Botrytis cinerea*

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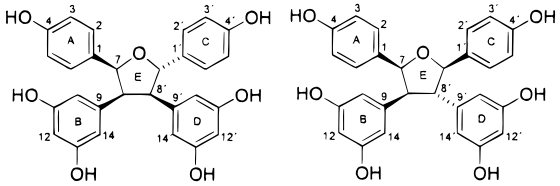
Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is produced by grapes (*Vitis* spp.) in response to microbial attack by the fungal grapevine pathogen *Botrytis cinerea*. Several reports indicate that pathogenic *B. cinerea* strains are capable of biotransforming resveratrol into an assortment of unidentified oxidized metabolites as a means of reducing the antifungal effects of resveratrol and facilitating *Botrytis* invasion into host-plant tissues. Studies utilizing growing incubations of *Botrytis cinerea* ATCC 11542 with resveratrol resulted in the production of three new (restrytisols A−C) (**1**−**3**) and three known (resveratrol *trans*-dehydrodimer, leachinol F, and pallidol) oxidized resveratrol dimers. All of the metabolites were evaluated for their anti-HIV-1, cytotoxic, and cyclooxygenase (COX) I and COX II inhibitory activities.

Grapes (*Vitis* spp.) produce the phytoalexin resveratrol (*trans*-3,4′,5-trihydroxystilbene) when challenged by microbial attack.[1] Resveratrol is then transformed by the plant into several more potent antifungal agents.[2] In agricultural settings, one of the most prevalent microbes that elicit this defense response in *Vitis* spp. is the fungal grapevine pathogen *Botrytis cinerea*.[3] It has been shown[1] that resveratrol rapidly accumulates in healthy tissues immediately surrounding the site of *B. cinerea* infection, presumably slowing or halting the spread of the infection.

Although elevated levels of resveratrol in host-plant tissues may slow or inhibit the spread of *B. cinerea* infection, some highly pathogenic *B. cinerea* strains can circumvent this defense by detoxifying resveratrol through an oxidative process.[4,5] The pathogenicity of several *B. cinerea* strains to grapes is positively correlated with the production of blue-copper oxidases known as stilbene oxidases or laccases.[6] Laccases are polyphenol oxidases recognized for their ability to catalyze the oxidation and polymerization of numerous phenolic substrates.[7] Studies have shown[5] that resveratrol is rapidly and thoroughly transformed in *B. cinerea* culture-medium filtrates containing laccases. Researchers previously identified[8] one of these oxidized transformation products as the resveratrol *trans*-dehydrodimer, while noting the presence of several other unidentified metabolites. Another study,[9] utilizing a purified laccase obtained from a *B. cinerea* isolate, suggested that a different oxidized dimer, ϵ-viniferin, was formed.

We initiated this study in order to investigate more thoroughly the *B. cinerea* transformation products of resveratrol. The identity of these metabolites is of special interest for several reasons. *B. cinerea* is a common grapevine pathogen causing significant losses for vineyard owners worldwide. By identifying the transformation products of resveratrol, we hope to learn more about the means utilized by pathogenic *B. cinerea* strains to detoxify resveratrol in the hope that selective fungicides could be utilized to target these pathways. In addition, we are interested in using microorganisms both as in vitro models to mimic and predict the metabolic fate of resveratrol in mammalian systems and as biocatalysts to prepare new resveratrol analogues that may possess significant biological activity.[10] The present study describes the isolation and structure elucidation of three new compounds, restrytisols A−C (**1**−**3**), and three known compounds [resveratrol *trans*-dehydrodimer (**4**), leachinol F, and pallidol]—all oxidized resveratrol dimers, following the incubation of resveratrol with growing *Botrytis cinerea* ATCC 11542 cultures.



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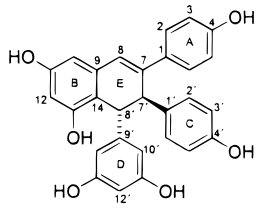

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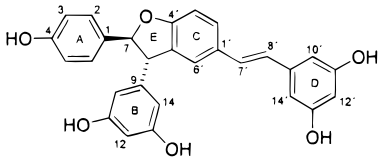

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Results and Discussion

Three *B. cinerea* strains (ATCC 11542, ATCC 12481, and ATCC 20599) and two *Botrytis allii* strains (ATCC 9435 and ATCC 42381) were screened for their ability to biotransform resveratrol. *B. cinerea* ATCC 11542 was selected for further investigation due to the thorough nature with which it metabolized resveratrol and the complexity of its metabolite profile as determined by TLC analysis. Further investigation indicated that a culture medium formulation composed of potato−dextrose broth supplemented with 2 g/L of pectin[11] would serve as an ideal

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medium for these studies due to the rapidity with which the transformation proceeded.

Resveratrol was incubated for 2 days with stage II cultures of *B. cinerea* ATCC 11542. The medium was extracted with ethyl acetate, and the extract was subjected to repeated column chromatography and preparative TLC with a variety of solvent systems, yielding six oxidized resveratrol dimers.

The negative ESIMS of restrytisol A (**1**) exhibited a prominent molecular ion at *m*/*z* 471 $[M - H]^-$ as the base peak; however, *m*/*z* 473 $[MH]^+$ was less apparent in the positive ESIMS, yielding, instead, a base peak of *m*/*z* 455 resulting from the loss of H_2O. Initial inspection of the ^1H NMR spectrum revealed several aromatic resonances, while the ^{13}C NMR spectrum exhibited 15 aromatic spins similar to those observed in resveratrol, indicating that **1** was an oxidized dimer with a molecular formula of $C_{28}H_{24}O_7$.

Closer examination of the aromatic region of the ^1H NMR spectrum showed that **1** contained 14 aromatic hydrogens, which were assigned to their respective carbons by means of a HETCOR experiment. Further analysis of the proton splitting patterns and DQF−COSY correlations revealed the presence of two 1,3,5-trisubstituted resorcinol moieties and two para-substituted phenols. All quaternary centers associated with these four substituted aromatic ring systems were then assigned based on long-range C−H FLOCK (J = 8.5, 9.6 Hz) correlations.

On TLC plates, resveratrol exhibits an intense purple color under UV irradiation (254 nm) due to the stilbene moiety's extended conjugation. In contrast, **1** was only slightly visible as a darkened zone under these same conditions. This information, coupled with the presence of four aliphatic methine carbons indicated a loss of conjugation in **1** after dimerization. The four remaining aliphatic proton resonances were then assigned to these carbons by means of HETCOR correlations. The vicinal coupling patterns of these four protons at δ 5.82 (d, 4.4 Hz, H-7), 3.84 (dd, 4.4, 6.2 Hz, H-8), 3.95 (dd, 6.4, 10.4 Hz, H-8′), and 5.51 (d, 10.3 Hz, H-7′) indicated that these hydrogens were coupled to one another in the order listed above. These correlations were confirmed by analysis of the DQF−COSY spectrum. Due to the downfield shift of C-7 and C-7′ and the presence of an additional oxygen (MS), it was concluded that the aliphatic methine carbons were arranged in a tetrahydrofuran moiety. Additional FLOCK correlations were then utilized to establish the connectivities of the aromatic substituents (rings A, B, C, and D) to the tetrahydrofuran ring (ring E). Significant correlations that were useful for determining these connectivities included those observed between C-2, C-6 and H-7; C-2′, C-6′ and H-7′; C-8 and H-10, H-14, as well as, C-8′ and H-10′, H-14′. Thus, the phenol moieties (rings A and C) were attached to C-7 and C-7′, respectively, while the resorcinol substituents (rings B and D) were attached to C-8 and C-8′, respectively.

A stereoisomer of **1** named tricuspidatol A had been isolated previously from the stem wood of *Parthenocissus tricuspidata* (Siebold & Zucc.) Planchon.[12] Tricuspidatol A exhibited only 10 carbon resonances, indicating that it was a highly symmetrical compound. Based on this fact, and the observance of W-type coupling among the protons of the tetrahydrofuran ring, tricuspidatol A was concluded to possess a trans−trans−trans relative configuration among the four tetrahydrofuran hydrogens. In contrast, we observed four unique spins in both the ^1H and ^{13}C NMR spectra for each of the four tetrahydrofuran carbons and hydrogens, which indicated that **1** was an asymmetric stereoisomer of tricuspidatol A. Due to the unreliable nature of coupling constants as indicators of stereochemistry in saturated five-membered ring systems, we employed difference NOE spectroscopy as a means of determining the relative stereochemistry of **1**.

All proton resonances in **1** were sequentially irradiated in a series of difference NOE experiments, and the magnitude of the resultant NOE enhancements recorded as strong (≥ 10%), moderate (5−9%), or weak (<4%). Upon irradiation of H-7, strong NOEs were observed for H-8 and H-8′, although a weak NOE appeared corresponding to H-2′, H-6′. Further irradiation of H-8 resulted in a strong NOE enhancement with H-7 and a moderate NOE with H-8′. Additional irradiation of H-8′ produced a strong NOE with H-7 and moderate NOEs with H-8 and H-2′, H-6′. Finally, when H-7′ was irradiated a strong NOE with H-10′, H-14′ was observed. In light of these results, a cis−cis−trans configuration was determined for H-7, H-8, H-8′, and H-7′, respectively. Therefore, the relative stereochemistry of restrytisol A was resolved as that indicated for structure **1**.

The positive ESIMS of restrytisol B (**2**) was similar to that obtained for **1**, with a molecular ion of *m*/*z* 473 $[MH]^+$ and a prominent base peak of *m*/*z* 455 resulting from the loss of H_2O. Accordingly, a molecular formula of $C_{28}H_{24}O_7$ was assigned to **2**.

Upon initial examination of the ^{13}C NMR spectrum of **2**, a striking similarity was noted with the chemical shifts of the spins observed in **1**. This suggested that **2** was a stereoisomer of **1**. This conjecture was confirmed upon examination of the ^1H NMR spectrum of **2**, which presented a similar set of aromatic proton resonances indicative of the presence of two 1,3,5-trisubstituted resorcinol moieties, two para-substituted phenols, and four aliphatic protons, which were all correlated by means of a DQF−COSY experiment. The aliphatic hydrogens of **2** exhibited chemical shifts similar to those found in ring E of **1**; however, the splitting patterns of H-7 (d, 8.8 Hz), H-8 (t, 9.2 Hz), H-7′ (d, 9.5 Hz), and H-8′ (t, 9.5 Hz) differed considerably. Two triplet-like spins at H-8 and H-8′ were in conflict with the expected splitting pattern for a tetrahydrofuran moiety. further examination of these resonances using homonuclear *J*-resolved spectroscopy indicated that the triplet-like character of H-8 and H-8′ were each the result of a coincidental overlap of a doublet of doublets.

Long-range FLOCK correlations (J = 8.5 Hz) were used to establish the connectivities of the four aromatic ring systems (rings A, B, C, and D) to the tetrahydrofuran moiety (ring E) in **2**. Significant complementary correlations between ring A and C-7/H-7, ring B and C-8/H-8, ring C and C-7′/H-7′, and ring D and C-8′/H-8′ facilitated the assignment of all proton and carbon spins in **2**.

Once again, the relative stereochemistry of the ring E chiral centers in **2** could not be accurately predicted based on coupling constant data; therefore, difference NOE experiments were utilized in order to address this issue. Irradiation of H-8 resulted in strong NOE enhancements for H-7 and H-10′, H-14′ and a moderate NOE for H-7′. Subsequent irradiation of H-7 produced moderate NOEs with H-8 and H-7′ and a small NOE with H-10′, H-14′. Further irradiation of H-7′ resulted in a strong NOE for H-10′, H-14′; a moderate NOE for H-7; and a weak NOE with H-8. Finally, when H-8′ was irradiated a strong NOE was observed for H-10, H-14 and moderate NOE enhancements for H-2, H-6 and H-2′, H-6′. As a result of these experiments a cis−trans−trans configuration is proposed

for H-7, H-8, H-8′, and H-7′, respectively, in restrytisol B as illustrated in **2**.

The HRESIMS of restrytisol C (**3**) exhibited a molecular ion of *m*/*z* 455.1491 $[MH]^+$, indicating that it was also a resveratrol dimer, with a molecular formula of $C_{28}H_{22}O_6$. This was confirmed upon examination of the ^1H and ^{13}C NMR spectra, which featured several aromatic spins characteristic of those previously observed in resveratrol. Despite the overlap of two pairs of spins at δ 6.28 (H-12 and H-12') and 6.84 (H-8 and H-3', H-5') in the 1D ^1H NMR spectrum, all proton resonances were resolved by means of a homonuclear *J*-resolved experiment. Subsequently, DEPT and HETCOR experiments were used to determine the multiplicities of all the carbon spins and to assign these to their respective protons. These experiments indicated that **3** contained 11 unique aromatic quaternary carbons, two aliphatic methines, and nine unique aromatic methines. The presence of only two aliphatic methines, in addition to this compound's intense purple color under UV irradiation (254 nm) (on TLC plates), indicated that **3** retained some portion of the extended conjugated system in resveratrol.

Further analyses utilizing DQF−COSY, FLOCK (J = 10.0 Hz), and COLOC (J = 8.0, 10.0 Hz) correlation experiments permitted the identification of one 1,3,5-trisubstituted resorcinol moiety (ring D) and two para-substituted phenols (rings A and C) in **3**. The remaining carbon resonances were composed of two olefinic spins at δ 123.6 (C-8, methine) and 138.7 (C-7, quaternary carbon), one 1,2,3,5-tetrasubstituted resorcinol moiety (ring B), and two aliphatic methines at δ 49.8 (C-7′) and 54.0 (C-8′). Due to the mutual coupling of the aliphatic methine protons H-7′ (J = 4.0 Hz) and H-8′ (J = 4.4 Hz), as observed in the DQF−COSY spectrum, the methine carbons C-7′ and C-8′ were concluded to be attached to one another. In light of the $^{2-3}J_{C-H}$ FLOCK and COLOC correlations, the degree of unsaturation required for this compound, and biogenic considerations, these fragments were cyclized to form ring E of the dihydronaphthalene nucleus in **3**. Additional long-range C−H (FLOCK and COLOC) correlations were then used to assign the aromatic substituents to their respective ring E carbons as depicted for compound **3**.

The relative stereochemistry of the chiral centers (C-7′ and C-8′) in **3** was addressed using difference NOE spectroscopy. Upon irradiation of H-7′, strong NOE enhancements were observed with H-2, H-6 and H-10′, H-14′ and moderate NOEs with H-2′, H-6′ and H-8′. Subsequent irradiation of H-8′ resulted in a moderate NOE with H-7′ and strong NOEs corresponding to H-2′, H-6′ and H-10′, H-14′. Thus, a trans relative stereochemistry was assigned for rings C and D in restrytisol C as shown in **3**. In further support of this conclusion, comparisons were made between the energy-minimized cis and trans conformers of **3** obtained using Insight II, Discover 95.0/3.0.0 modeling software on a Silicon Graphics workstation. The results of this comparative analysis indicated that in the minimized trans conformer of **3**, rings C and D would assume pseudoaxial positions thereby allowing for all the NOE enhancements observed in **3** to occur. In contrast, minimization of the cis isomer of **3** yielded two potential conformers. In both cases, one of the aromatic rings (C or D) and one of the aliphatic protons (H-8′ or H-7′, respectively) of the adjacent chiral center would assume trans pseudoaxial positions relative to each other. In cis conformer I, the trans pseudoaxial positions of ring C and H-8′ would prohibit the occurrence of the NOE enhancement between H-8′ and H-2′, H-6′, which was observed in **3**. Similarly, in cis conformer II, the trans pseudoaxial positions of ring D and H-7′ would prohibit the occurrence of the observed NOE enhancement between H-7′ and H-10′, H-14′ in **3**.

Comparisons of the observed ^1H and ^{13}C NMR data with values reported in the literature[8] indicated that **4** was the previously identified resveratrol *trans*-dehydrodimer. It was noted that three quaternary carbons (C-1, C-1′, and C-5′) had previously not been assigned and that ambiguity remained concerning the relative stereochemistry of the aliphatic dihydrofuran chiral centers of ring E (C-7 and C-8) in **4**.

The three quaternary centers in **4** were assigned using long-range C−H FLOCK (J = 5 Hz) and COLOC (J = 5 Hz) correlations. Significant two- and three-bond correlations were observed between δ 131.7 and H-8 and the protons of ring A. In contrast, δ 131.4 exhibited a two-bond correlation with H-8 and a three-bond correlation to H-3′, while δ 130.9 presented an important two-bond correlation with H-7′ and a three-bond correlation to H-3′. Thus, carbons δ 131.7, 131.4, and 130.9 were unambiguously assigned as C-1, C-5′, and C-1′, respectively.

Difference NOE experiments were used to establish the relative stereochemistry of the chiral centers in **4**. Strong NOEs were noted between H-7 and H-10, H-14, as well as between H-8 and H-2, H-6. Therefore, the aromatic substituents (rings A and B) of ring E in **4** were concluded to be arranged in a trans-configuration.

Two additional known resveratrol dimers, leachinol F[13] (in a 1:0.3 mixture with its C-7 epimer leachinol G) and pallidol,[14] were also isolated. The physical and spectral data of these compounds were in agreement with values reported in the literature. This represents the first report of leachinol F and pallidol as fungal metabolites of resveratrol.

Based on prior studies[5,8] demonstrating that resveratrol is oxidized in the presence of *B. cinerea* laccase preparations and the oxidized nature of the metabolites obtained in this study, we suspect that these dimers are the products of laccase catalysis. Previously, Pezet[9] asserted that a single oxidized dimer, ϵ-viniferin, is formed as the sole product of the catalysis of resveratrol by a laccase-like stilbene oxidase from a *B. cinerea* isolate. However, the use of a different *B. cinerea* strain (ATCC 11542) and culture conditions in our study afforded six different dimers, suggesting that other laccases or laccase isoforms[15] may also be involved in the oxidative dimerization of resveratrol.

The isolation of these six resveratrol dimers from growing *B. cinerea* cultures lends support to the accumulated evidence[6] that phytoalexin detoxification plays an important role in *B. cinerea* grapevine pathogenesis. However, dimer **4**, the major metabolite observed in this study, has been previously shown[16,17] to exhibit in vitro antifungal activity; therefore, further metabolism of these stilbene dimers may be necessary for their complete detoxification to be realized. Recently, intracellular *B. cinerea* laccases have been implicated as playing important roles in resveratrol detoxification, with the resultant metabolites sequestered in the conidia vacuoles where they would apparently undergo further metabolism.[4] A preliminary time−course TLC analysis of the transformation of resveratrol by *B. cinerea* ATCC 11542 showed that the dimers began to form within 24 h, peaked in intensity between 48 and 72 h, and then were gradually eliminated from the culture media until they were nearly undetectable by day 7. Therefore, these resveratrol dimers may represent the first in a series of detoxification steps employed by patho-

genic *B. cinerea* strains during the invasion of their *Vitis* spp. hosts.

All of the metabolites were evaluated for their anti-HIV-1, cytotoxic, and COX I and COX II inhibitory activities. None of the metabolites was found to inhibit viral proliferation in the anti-HIV-1 assay. Pallidol and **4** exhibited modest cytotoxicity against CEM (human lymphoblastoid) cells, with IC_{50} values of 32 and 49 μM, respectively. Although none of the metabolites inhibited COX II, **4** and **3** showed modest inhibitory activity against COX I, with IC_{50} values of 26 and 47 μM, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined in open capillary tubes with a Thomas−Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin−Elmer 241 digital polarimeter. IR spectra were recorded in KBr using a Nicolet Impact 400D FT−IR spectrophotometer. Centrifugation was carried out using a Heraeus Megafuge 2.0R centrifuge at 4 °C and 3600 × *g*. The term *in vacuo* refers to removal of solvent with a rotary evaporator under water aspirator vacuum (15−30 mmHg). All NMR spectra were obtained using acetone-d_6 on a JEOL-Eclipse 400 FT−NMR spectrometer. Standard pulse sequences were used for ^{1}H, ^{13}C, DQF−COSY, HETCOR, DEPT, COLOC, FLOCK, homonuclear *J*-resolved spectroscopy, and difference NOE experiments. LRESIMS analyses were conducted at the Mass Spectrometry Facility, Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195, using a Micromass Quattro II Tandem Quadrupole mass spectrometer. HRESIMS data were obtained using a Bruker BioAPEX 30es High-resolution FT mass spectrometer at the Department of Pharmacognosy, University of Mississippi.

Chromatographic Conditions. TLC analyses were performed on precoated Silica 60 UV_{254} plates (E. Merck, Darmstadt, Germany) and precoated Alugram RP_{18} F_{254} plates (Macherey−Nagel, Düren, Germany). Visualization of TLC plates was performed using 10% phosphomolybdic acid in absolute EtOH as a spray reagent. Spots were visualized by spraying the plates and then heating them at 110 °C for 1−2.5 min in an oven. Preparative TLC separations were carried out on 20 × 20 cm plates precoated with Si gel 60 F_{254} (2 mm layer thickness) (E. Merck) or Si gel C_{18} F_{254} (1 mm layer thickness) (Macherey−Nagel). Visualization of preparative TLC plates was performed under UV light (λ 254 nm). Column chromatography was performed using Si gel 60 (EM Science), C_{18} Si gel (Aldrich, Milwaukee, WI), and lipophilic Sephadex LH-20 (Sigma, St. Louis, MO). All solvents were of reagent grade quality or better.

Microorganisms. The *Botrytis* spp. utilized in this study were obtained from the American Type Culture Collection (Manassas, VA). Stock cultures of *Botrytis allii* ATCC 9435 were maintained on potato−carrot agar, while *Botrytis allii* ATCC 42381 and *Botrytis cinerea* strains (ATCC 11542, ATCC 12481, and ATCC 20599) were maintained on potato−dextrose agar (Difco, Detroit, MI) slants at 4 °C.

Media. All preliminary screening experiments were carried out in a medium consisting of dextrose (20 g), yeast extract (5 g), peptone (5 g), NaCl (5 g), K_2HPO_4 (5 g), and distilled H_2O (1000 mL). Subsequently, the growth of *B. cinerea* ATCC 11542 was tested in additional medium formulations consisting of potato−dextrose broth (Difco) with assorted additives (malt extract, gallic acid, pectin, EDTA, aqueous cucumber extract, K_2HPO_4, KH_2PO_4, and Na_2HPO_4). After examining several formulations, it was concluded that potato−dextrose broth (24 g/L) supplemented with 2 g/L of pectin (Spectrum, Gardena, CA) provided the best growth conditions for preparative-scale biotransformations with this organism.

Fermentation Procedures. Microbial metabolism studies were carried out by incubating the cultures with shaking on an Innova 5000 Digital Tier Shaker (New Brunswick Scientific Co., NJ), operating at 150 rpm and 25 °C. Preliminary screening experiments were carried out in 125-mL foam-plugged culture flasks containing 25 mL of medium. The media were sterilized at 121 °C and 18 psi for 15 min. Fermentations were carried out according to a standard two-stage protocol. In general, 5 mg of resveratrol was added in DMF (0.1 mg/μL) to the incubation media 72 h after inoculation of stage II cultures. The fermentations were sampled at 24-h intervals by extracting 3 mL of the culture broth with 3 × 3 mL EtOAc. The extracts were concentrated in vacuo and chromatographed on TLC plates. Substrate controls were composed of sterile media to which resveratrol was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions without the addition of resveratrol. Substrate-autoclaved culture controls consisted of microbial cultures that were grown for 3 days, autoclaved for 30 min, and then incubated after the addition of resveratrol.

Biotransformation of Resveratrol. Resveratrol was obtained from Pharmascience Inc. (Montreal, QC). The physical and spectral data of resveratrol have been previously reported in the literature.[18] A standard two-stage fermentation protocol was followed in carrying out the bioconversion of resveratrol. Two-liter flasks, each containing 400 mL of sterile medium, were inoculated with 5 mL of 72-h-old stage I *B. cinerea* culture and incubated on a shaker for 72 h. Portions (80 mg in 800 μL DMF) of resveratrol (6.04 g total) were added to each flask, after which the cultures were incubated for a period of 48 h with shaking. All flasks were covered with aluminum foil to prevent the UV-induced isomerization of resveratrol during incubation.

Isolation of Metabolites. After incubation, 400-mL aliquots of the growing cultures were placed in 1-L flasks and extracted by adding 250 mL of EtOAc to the flasks and shaking for 30−40 min on a rotary shaker. These aliquots were pooled and then centrifuged under the previously outlined conditions. The organic layer was aspirated, dried over Na_2SO_4, filtered, and evaporated in vacuo after which the process was repeated two additional times. A total of 5.8 g of crude, brown, gummy extract was obtained.

The crude extract was adsorbed onto a Si gel column and eluted with toluene−EtOAc−acetic acid (5:4.5:0.5) yielding two dark, oily fractions containing the metabolites. Fraction 1 (1.5 g) was subjected to repeated column chromatography on Si gel 60 with toluene−EtOAc−acetic acid (5:4.5:0.5) and $CHCl_3$−MeOH (6:1) and Sephadex (MeOH), yielding 101 mg (1.68% yield) of **4** as an amber solid. Fraction 2 (3.5 g) was again adsorbed onto a Si gel 60 column and eluted with toluene−EtOAc−acetic acid (6:3.5:0.5) yielding two fractions: fraction 2A (golden oil, 460 mg) and fraction 2B (brown foamy solid, 1.3 g). Fraction 2A was subjected to repeated preparative TLC on Si gel 60 with $CHCl_3$−MeOH (4:1 and 3:1), Si gel C_{18} preparative TLC with acetone−acetic acid−H_2O−EtOAc (2.5:0.5:7:0.8), Si gel C_{18} column chromatography using acetonitrile−H_2O (3:7), and Sephadex column chromatography with MeOH and MeOH−$CHCl_3$ (1:1) yielding 12 mg of **3** (0.2% yield) and 10 mg of pallidol (0.17% yield), both as white solids. Fraction 2B was adsorbed onto Si gel 60 and subjected to repeated column chromatography with toluene−EtOAc−acetic acid (4.5:4.5:1), $CHCl_3$−EtOAc−acetic acid (4.5:4.5:1), and CH_2Cl_2−MeOH (5:1); Si gel 60 preparative TLC using toluene−EtOAc−acetic acid (4.5:4.5:1), $CHCl_3$−EtOAc−acetic acid (5:4:1), and CH_2Cl_2−MeOH (5:1); Si gel C_{18} preparative TLC with acetonitrile−acetic acid−water (2.5:0.5:7) and acetone−acetic acid−water (2.5:0.5:7); and Sephadex column chromatography (MeOH), affording 11 mg (0.18% yield) of **1**, 27 mg (0.43% yield) of **2**, and 15 mg (0.24% yield) of leachinol F, each as a white solid. Spectral and physical data of leachinol F[13] and pallidol[14] were in agreement with those reported in the literature.

Restrytisol A (1): mp 117−119 °C; $[\alpha]^{25}_D$ −2.20° (*c* 1.8, acetone); IR (KBr) ν_{max} 3389, 1696, 1622, 1522, 1461, 1240, 1159 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; ESIMS *m/z* 471 $[M - H]^-$; HRESIMS *m/z* 495.1441 $[M + Na]^+$ (calcd for $C_{28}H_{24}NaO_7$ 495.1420).

Table 1. ^{1}H NMR Data (400 MHz) in Me_2CO-d_6 for Compounds **1-3** (δ, J in Hz)

	compound		
position	**1**	**2**	**3**
2, 6	7.17 d (8.4)	7.09 d (8.4)	6.34 d (8.0)
3, 5	6.64 d (8.4)	6.62 d (8.4)	6.41 d (8.0)
7	5.82 d (4.4)	5.49 d (8.8)	
8	3.84 dd (4.4, 6.2)	3.97 t (9.2)	6.84 br s[a]
10	6.11 d (2.3)	6.06 d (2.2)	6.00 br s
12	5.95 t (2.2)	5.96 t (2.0)	6.28 d (2.2)[b]
14	6.11 d (2.3)	6.06 d (2.2)	
2′, 6′	7.28 d (8.4)	7.31 d (8.4)	7.26 d (8.1)
3′, 5′	6.77 d (8.8)	6.82 d (8.4)	6.84 d (8.0)[a]
7′	5.51 d (10.3)	5.00 d (9.5)	4.79 d (4.0)
8′	3.95 dd (6.4, 10.4)	3.41 t (9.5)	4.62 d (4.4)
10′, 14′	6.08 d (2.2)	6.26 d (2.2)	6.50 d (2.2)
12′	6.05 t (2.2)	6.18 t (2.0)	6.28 t (2.2)[b]
OHs	7.80 br s	8.19 br s	8.06 br s
	8.00 br s	8.63 br s	8.10 br s
	8.25 br s		8.19 br s
			8.30 br s

[a,b] Overlapping spins were resolved by a homonuclear J-resolved NMR experiment.

Table 2. ^{13}C NMR Data (100 MHz) in Me_2CO-d_6 for Compounds **1-3** (δ, multiplicity[a])

	compound		
position	**1**	**2**	**3**
1	131.3 s	131.7 s	130.0 s
2, 6	127.3 d	128.1 d[c]	130.4 d
3, 5	114.4 d	114.2 d	113.9 d
4	155.6 s	155.9 s	155.8 s
7	84.2 d	83.6 d	138.7 s
8	57.6 d	59.2 d	123.6 d
9	139.8 s[b]	142.6 s	148.9 s
10	109.4 d	108.0 d	105.2 d[d]
11	157.5 s	157.7 s	156.3 s
12	100.6 d	100.5 d	101.4 d
13	157.5 s	157.7 s	150.9 s
14	109.4 d	108.0 d	124.7 s
1′	134.5 s	131.5 s	134.5 s
2′,6′	127.5 d	128.1 d[c]	129.7 d
3′,5′	115.0 d	115.0 d	114.9 d
4′	156.8 s	157.0 s	155.6 s
7′	82.3 d	86.6 d	49.8 d
8′	60.4 d	59.8 d	54.0 d
9′	139.8 s[b]	142.5 s	145.3 s
10′, 14′	107.7 d	106.8 d	105.2 d[d]
11′, 13′	157.8 s	158.5 s	158.7 s
12′	100.9 d	101.2 d	101.2 d

[a] Multiplicities were determined by DEPT experiment. [b−d] Overlapping spins.

Restrytisol B (2): mp 119−121 °C; $[\alpha]^{25}_D$ −0.42° (*c* 4.7, acetone); IR (KBr) ν_{max} 3402, 1696, 1622, 1521, 1454, 1246, 1159 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; ESIMS *m/z* 473 $[MH]^+$; HRESIMS *m/z* 495.1430 $[M + Na]^+$ (calcd for $C_{28}H_{24}NaO_7$ 495.1420).

Restrytisol C (3): mp 133−136 °C; $[\alpha]^{25}_D$ −0.92° (*c* 1.1, acetone); IR (KBr) ν_{max} 3416, 1716, 1702, 1622, 1515, 1461, 1240, 1159 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 455.1491 $[MH]^+$ (calcd for $C_{28}H_{23}O_6$ 455.1494).

Resveratrol *trans*-dehydrodimer (4): mp 150−152 °C; $[\alpha]^{25}_D$ −1.15° (*c* 7.3, acetone); IR (KBr) ν_{max} 3342, 1602, 1488, 1347, 1240, 1152 cm^{-1}; ^{1}H and ^{13}C NMR data were in agreement with those reported in the literature[8] (values for melting point, optical rotation, and IR have not been previously reported[8,16]).

Biological Assays. Determinations of anti-HIV-1 activity in PBM (human peripheral blood mononuclear) and cytotoxicity against CEM, PBM, and Vero (African green monkey) cells were performed as previously described.[19] Enzyme inhibition studies of COX I and COX II were carried out using previously described protocols.[20, 21]

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Supporting Information Available: Figures providing detailed long-range C−H correlations and difference NOE enhancements for restrytisols A−C and depictions of the molecular modeling studies of restrytisol C are available. The structures of leachinol F and pallidol are also given. These materials can be obtained free of charge via the Internet at http://pubs.acs.org.

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