Microbial Transformations of Aryltetralone and Aryltetralin Lignans by *Cunninghamella echinulata* and *Beauveria bassiana*

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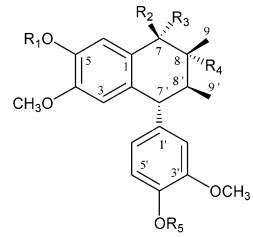
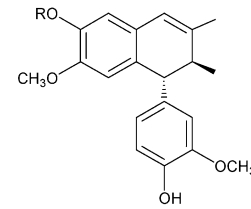
Microbiological transformation of the aryltetralone lignan (−)-8′-*epi*-aristoligone (**1**) with *Cunninghamella echinulata* ATCC 10028B afforded two known natural lignans, (−)-holostyligone (**3**) and (−)-arisantetralone (**4**). Incubation of the aryltetralin lignan (−)-isogalbulin (**2**), obtained by chemical transformation of **1**, with *C. echinulata* ATCC 10028B afforded the known lignan aryltetralol (**5**) and seven new metabolites, (−)-8-hydroxyisogalbulin (**6**), (−)-7-methoxyisogalbulin (**7**), (−)-4′-*O*-demethyl-8-hydroxyisogalbulin (**8**), (−)-7-methoxy-8-hydroxyisogalbulin (**9**), (−)-4′-*O*-demethyl-7-methoxyisogalbulin (**10**), (−)-4′,5-*O*-didemethylcyclogalgravin (**11**), and (−)-4′-*O*-demethylcyclogalgravin (**12**). When **2** was subjected to biotransformation with *Beauveria bassiana* ATCC 7159, (−)-8-hydroxyisogalbulin (**6**) was the only isolable product. The structures of all new compounds were established by detailed analysis of their spectroscopic data.

Lignans are a class of plant secondary metabolites with high structural diversity and are biogenetically derived by the oxidative dimerization of phenylpropenoids.[1] The current interest in lignans stems from their known medicinal potential as hepatoprotective,[2] antioxidative,[3] antiviral[4] including anti-HIV,[5] antibacterial,[6] antiparasitic,[7] antifungal,[8] anti-inflammatory,[9] and anticancer[10] agents. In addition, recent studies have shown that the presence of high levels of lignans in humans reduces the risks of prostate, ovarian, and breast cancers, osteoporosis, and cardiovascular disease.[11] Lignans, especially those belonging to the classes of aryltetralins and aryltetralones, have attracted attention due to the commercial importance of podophyllotoxin as a raw material for the semisynthesis of the anticancer drugs etoposide and teniposide.[12] Significant antiplasmodial activity of the aryltetralone lignan (−)-8′-*epi*-aristoligone (**1**)[13,14] and its potential as an antimalarial drug together with its ready availability from *Holostylis reniformis* Duch. (Aristolochiaceae)[13] prompted us to investigate the application of microbial biotransformation of **1** and the aryltetralin lignan (−)-isogalbulin (**2**), obtained by chemical transformation of **1**, to generate their novel analogues for future biological testing. Although there are a number of reports on metabolism of phenolic tetralin and furofuranoid lignans including pinoresinol,[15] deoxypodophyllotoxin,[16] and non-phenolic furofuranoid lignans (+)-eudesmin,[15] (+)-magnolin,[15] and (+)-yangabin[15] by microbes, there are no reports on microbial biotransformation of the non-phenolic aryltetralin and aryltetralone lignans. In continuing our studies on microbial biotransformation of natural products,[17] we have investigated the metabolism of **1** and **2** by *Cunninghamella echinulata* ATCC 10028B and *Beauveria bassiana* ATCC 7159, and here we report the isolation and characterization of seven new and three known analogues of these lignans.





1 $R_1 = R_5 = CH_3$, R_2 , $R_3 = O$, $R_4 = H$
2 $R_1 = R_5 = CH_3$, $R_2 = R_3 = R_4 = H$
3 $R_1 = CH_3$, R_2 , $R_3 = O$, $R_4 = R_5 = H$
4 $R_1 = R_4 = R_5 = H$, R_2 , $R_3 = O$
5 $R_1 = R_5 = CH_3$, $R_2 = R_4 = H$, $R_3 = OH$
6 $R_1 = R_5 = CH_3$, $R_2 = R_3 = H$, $R_4 = OH$
7 $R_1 = R_5 = CH_3$, $R_2 = R_4 = H$, $R_3 = OCH_3$
8 $R_1 = CH_3$, $R_2 = R_3 = R_5 = H$, $R_4 = OH$
9 $R_1 = R_5 = CH_3$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = OH$
10 $R_1 = CH_3$, $R_2 = R_4 = R_5 = H$, $R_3 = OCH_3$

11 R = H
12 R = CH_3

Results and Discussion

Incubation of the aryltetralone lignan (−)-8′-*epi*-aristoligone (**1**) with *C. echinulata* afforded two polar products identified as (−)-holostyligone (**3**)[13] and (−)-arisantetralone (**4**),[14] suggesting that the substrate has undergone stepwise regioselective de-*O*-methylations during the biotransformation. Although *C. echinulata* is known to effect de-*O*-methylation of alkaloids,[18,19] and regioselective de-*O*-methylation of lignans has been reported in biotransformation of (+)-eudesmin and (+)-magnolin with *Aspergillus niger*,[15] this constitutes the first report of de-*O*-methylation of a lignan by *C. echinulata*.

The known ability of *C. echinulata* to hydroxylate benzylic positions of aromatic substrates[20] prompted us to investigate the metabolism of aryltetralin lignans by this fungus. To this end, the aryltetralin lignan (−)-isogalbulin (**2**) was obtained in high overall yield by the treatment of (−)-8′-*epi*-aristoligone (**1**) with $NaBH_4$/MeOH followed by hydrogenolysis (H_2/10% Pd on C/EtOH). When **2** was incubated with *C. echinulata* for 20 days, the EtOAc extract of the resulting culture broth afforded the known lignan aryltetralol (**5**)[14] and seven new metabolites, **6**−**12**. Metabolite **6** was obtained as a white, amorphous solid and was determined to have the molecular formula $C_{22}H_{28}O_5$ by a combination of HRMS and ^{13}C NMR data. The ^1H NMR spectrum of **6** (Table 1) showed very close resemblance to that of (−)-isogalbulin (**2**), the major difference between them being due to the absence of one methine signal and one secondary methyl signal in **6**, and instead it showed the presence of a quarternary methyl group attached to an oxygenated carbon atom. The presence of this oxygenated aliphatic carbon in **6** was also inferred from its ^{13}C NMR spectrum (Table 2), which showed a signal at δ 72.2. These data suggested that an oxygenation had

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Table 1. ^{1}H NMR Data (δ) for Lignans **6**−**10** ($CDCl_3$, 400 MHz, *J* in Hz)

H	6	7	8	9	10
3	6.17 (s)	6.25 (s)	6.18 (s)	6.28 (s)	6.25 (s)
6	6.53 (s)	6.75 (s)	6.52 (s)	6.66 (s)	6.74 (s)
7α	2.81 (d, 15.2)		2.81 (d, 15.2)		
7β	3.04 (d, 15.2)	3.99 (d, 3.2)	3.03 (d, 15.2)	3.86 (s)	3.98 (d, 3.6)
8		2.36 (m)			2.35 (m)
9	1.16 (s)	0.85 (d, 6.8)	1.16 (s)	1.01 (s)	0.87 (d, 6.8)
2′	6.56 (d, 2.0)	6.58 (d, 2.0)	6.52 (d, 2.0)	6.60 (d, 2.0)	6.54 (d, 2.0)
5′	6.78 (d, 8.0)	6.77 (d, 8.0)	6.83 (d, 8.0)	6.78 (d, 8.0)	6.81 (d, 8.0)
6′	6.69 (dd, 8.0, 2.0)	6.67 (dd, 8.0, 2.0)	6.65 (dd, 8.0, 2.0)	6.71 (dd, 8.0, 2.0)	6.64 (dd, 8.0, 2.0)
7′	3.41 (d, 10.8)	3.39 (d, 10.0)	3.40 (d, 10.4)	3.32 (d, 10.4)	3.37 (d, 10.0)
8′	1.99 (m)	2.23 (m)	1.97 (m)	2.32 (m)	2.23 (m)
9′	0.88 (d, 6.4)	0.84 (d, 6.8)	0.88 (d, 6.8)	0.94 (d, 6.8)	0.84 (d, 6.8)
OCH_3-3′	3.78 (s)	3.76 (s)	3.79 (s)	3.78 (s)	3.76 (s)
OCH_3-4	3.56 (s)	3.59 (s)	3.57 (s)	3.60 (s)	3.60 (s)
OCH_3-4′	3.87 (s)	3.87 (s)		3.89 (s)	
OCH_3-5	3.84 (s)	3.85 (s)	3.84 (s)	3.86 (s)	3.87 (s)
OCH_3-7		3.44 (s)		3.46 (s)	3.44 (s)
OH			5.50 (s), 3.88 (s)		5.46 (s)

Table 2. ^{13}C NMR Data (δ) for **6** and **8**−**10** ($CDCl_3$, 100 MHz)

C	6	8	9	10
1	127.5 (s)	127.5 (s)	126.2 (s)	126.7 (s)
2	130.7 (s)	130.8 (s)	133.1 (s)	133.0 (s)
3	111.9 (d)	112.6 (d)	112.8 (d)	112.7 (d)
4	149.0 (s)	147.5 (s)	149.3 (s)	148.7 (s)
5	147.3 (s)	147.3 (s)	146.8 (s)	147.3 (s)
6	112.5 (d)	113.9 (d)	113.0 (d)	113.6 (d)
7	47.2 (t)	47.2 (t)	85.7 (d)	83.1 (s)
8	72.2 (s)	72.2 (s)	73.2 (s)	35.2 (d)
9	20.4 (q)	20.4 (q)	17.7 (q)	17.1 (q)
1′	138.2 (s)	137.6 (s)	138.6 (s)	137.9 (s)
2′	111.1 (d)	111.1 (d)	112.2 (d)	112.5 (d)
3′	147.5 (s)	146.7 (s)	148.9 (s)	146.6 (s)
4′	147.5 (s)	144.1 (s)	147.5 (s)	143.9 (s)
5′	110.8 (d)	111.0 (d)	110.7 (d)	111.2 (d)
6′	121.6 (d)	122.6 (d)	121.5 (d)	122.7 (d)
7′	53.1 (d)	53.2 (d)	53.1 (d)	49.2 (d)
8′	45.9 (d)	45.9 (d)	41.4 (d)	35.1 (d)
9′	12.6 (q)	12.6 (q)	12.3 (q)	11.3 (q)
OCH_3-3′	55.9 (q)	55.9 (q)	56.7 (q)	56.0 (q)
OCH_3-4	55.8 (q)	55.8 (q)	55.9 (q)	55.9 (q)
OCH_3-4′	55.8 (q)		55.8 (q)	
OCH_3-5	55.8 (q)	55.8 (q)	55.8 (q)	55.8 (q)
OCH_3-7			55.7 (q)	55.7 (q)

taken place at C-8 or C-8′ of the substrate to give an alcohol. The coupling pattern observed for the two geminal protons at C-7 (δ 2.44 and 2.84) of **2** had changed from two double doublets to two doublets (δ 2.81 and 3.04) during its biotransformation to **6**, suggesting that the oxygenation occurred at C-8. The methyl group at C-8 of **6** showed NOE correlations with the C-8′ methyl group and H-7′, confirming the β-orientation of these groups. Thus, this metabolite was identified as (−)-8-hydroxyisogalbulin [(7′*R*,8*S*,8′*S*)-8,8′-dimethyl-3′,4,4′,5-tetramethoxy-2,7′-cyclolignan-8-ol] (**6**). Although the synthesis of **6** has been reported previously,[21,22] none of these reports have defined the relative configuration at C-8 and/or C-8′.

The HRMS data for the metabolite **7** suggested the molecular formula $C_{23}H_{30}O_5$. Its ^{1}H NMR spectroscopic data (Table 1) indicated the presence of 1,2,4,5-tetrasubstituted and 1,3,4-trisubstituted benzene rings, four aromatic OCH_3, one aliphatic OCH_3, four methine protons of which one is oxygenated [δ 3.99 (d, J = 3.2 Hz)], and two secondary CH_3 groups. Comparison of these data with those of aryltetralol (**5**) suggested that **7** had the same carbon skeleton; the major difference was found to be the presence of an OCH_3 instead of an OH group on an aliphatic carbon. In order to satisfy these data, this newly introduced OCH_3 was placed at C-7. The orientation of this OCH_3 group was determined to be α on the basis of NOE correlations observed between the oxymethine proton (H-7) and the methine proton at δ 3.39 (H-7′); the latter proton also showed a strong NOE correlation to CH_3-9′ at δ 0.84. The foregoing led to the identification of this metabolite as (−)-7-methoxyisogalbulin [(7*R*,7′*R*,8*S*,8′*S*)-8,8′-dimethyl-3′,4,4′,5,7-pentamethoxy-2,7′-cyclolignan] (**7**).

Metabolite **8**, obtained as a white, amorphous solid, was determined to have the molecular formula $C_{21}H_{26}O_5$ by a combination of its HRFABMS and ^{13}C NMR data. The ^{1}H NMR data of **8** very closely resembled those of **6**, the major difference between them being due to the absence of one aromatic OCH_3 group in **8**. Irradiation of the OCH_3 signals at δ_H 3.57 (OCH_3-4), 3.79 (OCH_3-3′), and 3.84 (OCH_3-5) showed enhancement of the signals at δ_H 6.18 (H-3), 6.52 (H-2′), and 6.52 (H-6), respectively (Figure 1), suggesting that these OCH_3 groups and aromatic protons are in close proximity to each other and the possible absence of an OCH_3 group adjacent to H-5′. These data also suggested that de-*O*-methylation had taken place at C-4′ of the C ring of the lignan. Metabolite **8** was thus identified as (−)-4′-*O*-demethyl-8-hydroxyisogalbulin [(7′*R*,8*S*,8′*S*)-8,8′-dimethyl-4′-hydroxy-3′,4,5-trimethoxy-2,7′-cyclolignan-8-ol].

The molecular formula of **9** was determined to be $C_{23}H_{30}O_6$ by a combination of HRFABMS and ^{1}H and ^{13}C NMR data. Its ^{1}H NMR spectrum was very similar to that of **7**, the major difference between them being due to the absence of one methine signal and one secondary CH_3 signal in **9** and instead the presence of one CH_3 group attached to an oxygenated quaternary carbon. These data suggested that an additional OH group was present in **9**. The oxymethine proton at δ 3.86 of **9** showed HMBC correlations to C-1, C-2, C-6, C-8, C-8′, and C-9 (Figure 2), placing it at C-7. The OCH_3 group at δ 3.46 showed HMBC correlations with the oxymethine carbon at δ 85.7, suggesting its attachment to C-7. Therefore, the additional OH group should be located at C-8 or

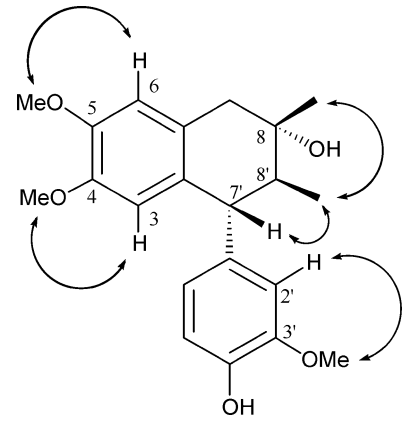

Figure 1. Selected NOE correlations of **8**.

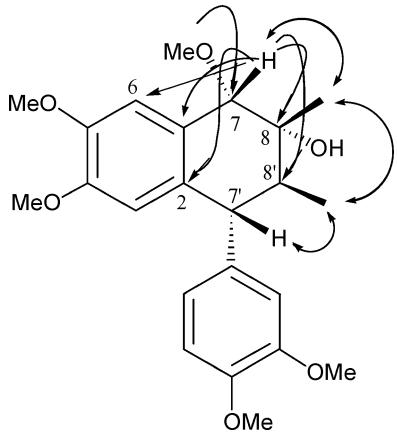

Figure 2. Selected HMBC (H→C) and NOE (H↔H) correlations of **9**.

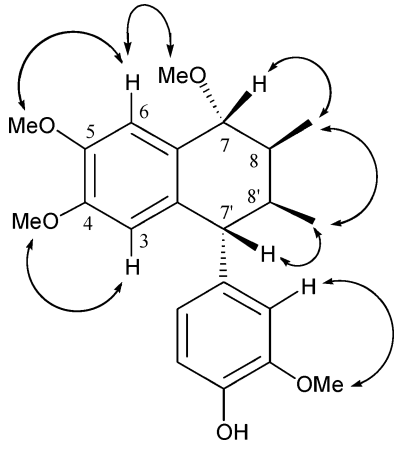

Figure 3. Selected NOE correlations of **10**.

C-8′. Since H-7′ appeared as a doublet (J = 10.4 Hz), this OH group should be located at C-8. The ^{1}H NMR singlet at δ 3.86 (H-7) showed an NOE correlation to the CH_3 signal at δ 1.01. Since the CH_3 groups are β-oriented, H-7 should have the same orientation. Thus, **9** was identified as (−)-7-methoxy-8-hydroxyisogalbulin [(7*S*,7′*R*,8*R*,8′*S*)-8,8′-dimethyl-3′,4, 4′,5,7-pentamethoxy-2,7′-cyclolignan-8-ol].

Metabolite **10**, obtained as a white, amorphous solid, was determined to have the molecular formula $C_{22}H_{28}O_5$ by a combination of HRFABMS and ^{13}C NMR data. The ^{1}H NMR data of **10** indicated the presence of five aromatic protons in two aromatic rings, three OCH_3 groups attached to olefinic/aromatic carbons, one OCH_3 group attached to an aliphatic carbon (δ 3.44), one oxymethine proton [δ 3.98 (d, J = 3.6 Hz)], three methine protons, and two secondary CH_3 groups (Table 1). Irradiation of the OCH_3 signals at δ_H 3.87, 3.76, 3.60, and 3.44 showed enhancement of those at δ_H 6.74 (H-6), 6.54 (H-2′), 6.25 (H-3), and 6.74 (H-6), respectively (Figure 3), suggesting that demethylation occurred at C-4′ and the OCH_3 group attached to the aliphatic carbon is located at C-7. The oxymethine proton at δ 3.98 showed NOE correlations with CH_3-8 protons, confirming its β-orientation. This compound was thus identified as (−)-4′-*O*-demethyl-7-methoxyisogalbulin [(7*R*,7′*R*,8*S*,8′*S*)-8,8′-dimethyl-4′-hydroxy-3′,4,5,7-tetramethoxy-2,7′-cyclolignan] (**10**).

Metabolite **11**, obtained as an off-white, amorphous solid, was determined to have the molecular formula $C_{20}H_{22}O_4$ by a combination of HRFABMS and ^{1}H and ^{13}C NMR data. Its ^{1}H NMR spectrum showed the presence of 1,2,4,5-tetrasubstituted and 1,3,4-trisubstituted benzene rings, an olefinic/aromatic proton (δ 6.09), two aromatic OCH_3 (δ 3.76 and 3.75), two methine protons [δ 3.62 (d, J = 3.2 Hz) and 2.32 (dq, J = 7.2, 3.2 Hz)], a CH_3 attached to an olefinic carbon [(δ 1.76 (s)], a secondary CH_3 group [1.05 (d, J = 7.2 Hz)], and two OH groups [δ 5.43 (s) and 5.40 (s); D_2O exchangeable]. The ^{13}C NMR spectrum of **11**, when analyzed in combination with the HSQC data, showed the presence of 14 aromatic/olefinic carbons, of which four were oxygenated (δ 146.2, 145.1, 144.2 and 143.8) and six were protonated (δ 121.1, 120.4, 113.9, 112.1, 111.7, and 110.1), two methine carbons, two OCH_3 carbons, and two CH_3 carbons. The aromatic/olefinic proton at δ 6.09 showed HMBC correlations with C-2 (δ 126.9), C-6 (δ 111.7), and C-8′ (42.1), placing it at C-7. The D_2O exchangeable singlet at δ 5.40 (OH) showed HMBC correlations with C-4 and C-6, locating it at C-5, and the other OH showed HMBC correlations with C-3′ and C-5′, placing it at C-4′ (Figure 4). This metabolite was thus identified as (−)-4′,5-*O*-didemethylcyclogalgravin [(7′*R*,8′*S*)- 4′,5-dihydroxy-8,8′-dimethyl-3′,4-dimethoxy-2,7′-cyclolignan-7-ene] (**11**).

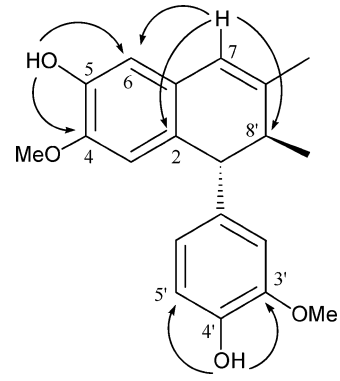

Figure 4. Selected HMBC correlations of **11**.

The molecular formula of metabolite **12** was determined to be $C_{21}H_{24}O_4$ from its HRMS data. The ^{1}H NMR spectroscopic data (see Experimental Section) of this compound closely resembled those of (−)-4′,5-*O*-didemethylcyclogalgravin (**11**) except for the presence of an additional aromatic OCH_3 group in **12**. The presence of a strong NOE of this OCH_3 to H-6 confirmed that it was located at C-5. Thus, this metabolite was identified as (−)-4′-*O*-demethylcyclogalgravin [(7′*R*,8′*S*)-8,8′-dimethyl-4′-hydroxy-3′,4,5-trimethoxy-2,7′-cyclolignan-7-ene] (**12**).

Experimental Section

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ with a JASCO Dip-370 digital polarimeter. 1D and 2D NMR spectra were recorded in $CDCl_3$ with a Bruker Avance III 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR using residual $CHCl_3$ resonances as internal reference. Low-resolution and high-resolution MS were recorded on Shimadzu LCMS-QP8000α and JEOL HX110A spectrometers, respectively. Analytical and preparative thin-layer chromatography (TLC) were performed on precoated 0.25 mm thick plates of silica gel 60 F_{254}; spraying with a solution of 7% phosphomolybdic acid in EtOH followed by heating was used to visualize the spots on analytical TLC. Preparative HPLC was performed on a Waters Delta Prep 4000 system equipped with a Waters 996 photodiode array detector and a Waters Prep LC controller utilizing Empower Pro software and using a RP column (Phenomenex Luna 5 μm, C_{18}, 100 Å, 250 × 10 mm) with a flow rate of 3.0 mL/min; chromatograms were acquired at 254 and 270 nm. (−)-8′-*epi*-Aristoligone (**1**) was isolated from *Holostylis reniformis* Duch. (Aristolochiaceae) as previously reported.[13]

Conversion of (−)-8′-*epi*-Aristoligone (1) to (−)-Isogalbulin (2). To a stirred solution of **1** (200 mg, 0.5 mmol) in MeOH (7 mL) at 0 °C was added $NaBH_4$ (100 mg, 2.6 mmol). After 1 h at 0 °C (TLC control) excess $NaBH_4$ was destroyed by adding ice. The resulting solution was evaporated under reduced pressure to remove MeOH and extracted with EtOAc (3 × 25 mL), and the organic extract was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to afford aryltetralol (**5**) (198 mg, 97%). Lignan **5** (195 mg, 0.5 mmol)

was dissolved in ethanol (50 mL), and 10% Pd on C (ca.10 mg) was added to the solution, which was stirred at room temperature under an atmosphere of H_2. After 2 h (TLC control), the reaction mixture was filtered and the filtrate evaporated under reduced pressure to afford **2** (175 mg, 94%) as a white solid.

Aryltetralol (5): white, amorphous powder; $[\alpha]^{25}_D$ −38.2 (*c* 0.41, $CHCl_3$) [lit.[14] −36.0 (*c* 0.83, $CHCl_3$)]; ^{1}H and ^{13}C NMR, and MS data were consistent with reported data.[14]

(−)-Isogalbulin (2): colorless needles (MeOH); mp 98−100 °C (lit.[23] 101−102 °C); $[\alpha]_D^{25}$ −35.8 (*c* 0.7, $CHCl_3$) [lit.[23] −36.7 (*c* 0.08, $CHCl_3$)]; ^{1}H NMR and MS data were consistent with reported data.[23]

Microbial Transformation of (−)-8′-*epi*-Aristoligone (1) by *C. echinulata* ATCC 10028B. *C. echinulata* was grown in a two-stage fermentation procedure in soybean meal/glucose medium [soybean meal (5 g), glucose (20 g), K_2HPO_4 (5 g), NaCl (5 g), yeast extract (5 g), distilled H_2O (1 L)]. The pH of the medium was adjusted to 7.0 and autoclaved at 121 °C for 15 min. Small-scale fermentations were performed in 125 mL Erlenmeyer flasks, each holding 25 mL of the culture medium on a rotary shaker operating at 220 rpm at 28 °C for 24 h. The substrate (**1**, 2.5 mg in 0.25 mL of DMF) was added to 24 h old second-stage culture. Culture control consisted of fermentation broth of *C. echinulata* without the substrate but with the same volume of DMF, and the substrate control consisted of soybean meal/glucose medium with the same amount of **1** in DMF. Both controls were incubated under the same conditions. Samples (4 mL each) were taken from all three flasks at various time intervals and individually extracted with EtOAc (4 mL), and extracts were examined for the disappearance of **1** by TLC. Large-scale biotransformation was performed under the same conditions but in 4 × 250 mL flasks holding 50 mL of the medium in each flask. A total of 21.1 mg of **1** was used, and after 20 days of incubation, the fermentation broths were combined, mycelia were removed by filtration and washed with H_2O (3 × 250 mL), and the washings were combined with the filtrate, neutralized with 1 M HCl, and extracted with EtOAc (3 × 200 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to give the EtOAc extract (20.1 mg) as a brown syrup. This was separated by preparative TLC (silica gel) using hexanes/EtOAc/$CHCl_3$ (6:3:1) to give **1** (2.0 mg, R_f 0.53), **3** (0.5 mg, R_f 0.40), and **4** (1.5 mg, R_f 0.22).

(−)-Holostyligone (3): colorless, amorphous solid; $[\alpha]^{25}_D$ −38.2 (*c* 0.05, $CHCl_3$) [lit.[14] −27.0 (*c* 1.18, $CHCl_3$)]; ^{1}H and ^{13}C NMR and MS data were consistent with reported data.[14]

Arisantetralone (4): colorless, amorphous solid; $[\alpha]^{25}_D$ −35.4 (*c* 0.05, $CHCl_3$) [lit.[24] −35.0 (*c* 0.1, $CHCl_3$)]; ^{1}H and ^{13}C NMR and MS data were consistent with reported data.[24]

Microbial Transformation of (−)-Isogalbulin (2) by *C. echinulata* ATCC 10028B. *C. echinulata* (ATCC 10028B) was grown in a two-stage fermentation procedure in a soybean meal/glucose medium. Small-scale microbial transformation of (−)-isogalbulin (**2**) by the organism was carried out in 125 mL Erlenmeyer flasks containing 50 mL of medium on a rotary shaker operating at 220 rpm and 28 °C. The substrate (**2**, 5.0 mg in 0.25 mL of DMF) was added to 24 h old second-stage culture. Culture control consisted of a fermentation broth of *C. echinulata* without the substrate but with the same volume of DMF, and the substrate control consisted of sterile soybean meal/glucose medium with the same amount of **2** in DMF. Both controls were incubated under the same conditions. Samples (4 mL each) were taken from all three flasks at various time intervals and extracted separately with EtOAc (4 mL), and extracts were examined for the formation of metabolites by TLC. A preparative-scale microbial transformation was performed under the same conditions in (5 × 1 L) Erlenmeyer flasks holding 250 mL of medium. A total of 150 mg of **2** was used. After 20 days mycelia were separated by filtration and combined supernatant was extracted with EtOAc (3 × 500 mL). The combined EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a crude extract (149.5 mg). This extract was then subjected to gel permeation chromatography over Sephadex LH-20 (4 g) made up in hexanes/CH_2Cl_2 (1:4) and eluted with hexanes/CH_2Cl_2 (1:4, 180 mL), CH_2Cl_2/acetone (3:2, 90 mL), CH_2Cl_2/acetone (1:4, 90 mL), CH_2Cl_2/MeOH (1:1, 90 mL), and finally MeOH (50 mL). Fractions (6 mL each) were collected, and those having similar TLC patterns were combined to give 14 major fractions (F_1−F_{14}). Further separation of fraction F_2 (41.4 mg) over a column of silica gel (1.5 g) made up in CH_2Cl_2, elution with CH_2Cl_2 containing increasing amounts of MeOH, and combining fractions having similar TLC patterns afforded three subfractions (F_{2A}−F_{2C}). Subfraction F_{2B} was then separated by RP-HPLC using 50% aqueous CH_3CN to give **5** (1.0 mg) and **6** (11.8 mg). Subfraction F_{2c} was also separated by RP-HPLC, but using 45% aqueous CH_3CN to give an additional quantity of **6** (3.5 mg) and **7** (6.0 mg). Fraction F_4 from the above Sephadex column was directly subjected to RP-HPLC using 50% aqueous CH_3CN to give **8** (3.6 mg), **9** (2.3 mg), and **11** (2.2 mg). RP-HPLC of F_5 using 70% aqueous CH_3CN gave an additional quantity of **8** (8.4 mg), and a crude fraction was separated by RP-HPLC using 60% aqueous CH_3CN to give **10** (1.3 mg). Fraction F_8 from the Sephadex column on further purification by RP-HPLC using 30% aqueous CH_3OH as eluant afforded **12** (1.8 mg).

(−)-8-Hydroxyisogalbulin (6): white, amorphous powder; $[\alpha]^{25}_D$ −15.6 (*c* 0.11, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRAPCIMS *m/z* 371.1862 $[M - H]^+$ (calcd for $C_{22}H_{27}O_5$, 371.1864).

(−)-7-Methoxyisogalbulin (7): white, amorphous powder; $[\alpha]^{25}_D$ −51.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR data, see Table 1; HRAPCIMS *m/z* 409.1987 $[M + Na]^+$ ($C_{23}H_{30}NaO_5$, 409.1985).

(−)-4′-*O*-Demethyl-8-hydroxyisogalbulin (8): white, amorphous powder; $[\alpha]^{25}_D$ −15.0 (*c* 0.16, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 357.1707 $[M - H]^+$ (calcd for $C_{21}H_{25}O_5$, 357.1707).

(−)-7-Methoxy-8-hydroxyisogalbulin (9): white, amorphous powder; $[\alpha]^{25}_D$ −34.7 (*c* 0.18, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 425.1936 $[M + Na]^+$ (calcd for $C_{23}H_{30}NaO_6$, 425.1935).

(−)-4′-*O*-Demethyl-7-methoxyisogalbulin (10): white, amorphous powder; $[\alpha]^{25}_D$ −16.1 (*c* 0.2, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRAPCIMS *m/z* 371.1862 $[M - H]^+$ (calcd for $C_{22}H_{27}O_5$, 371.1864).

(−)-4′,5-*O*-Didemethylcyclogalgravin (11): white, amorphous powder; $[\alpha]^{25}_D$ −110.5 (*c* 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (1H, d, J = 8.0 Hz, H-5′), 6.64 (1H, s, H-6), 6.56 (1H, d, J = 2 Hz, H-2′), 6.54 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.49 (1H, s, H-3), 6.09 (1H, s, H-7), 5.43 (1H, s, D_2O exchangeable, OH-4′), 5.40 (1H, s, D_2O exchangeable, OH-5), 3.76 (3H, s, OMe), 3.75 (3H, s, OMe), 3.62 (1H, d, J = 3.2 Hz, H-7′), 2.32 (1H, dq, J = 7.2, 3.2 Hz, H-8′), 1.76 (3H, s, H_3-9), 1.05 (1H, d, J = 7.2 Hz, H_3-9′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.2 (C, C-3′), 145.1 (C, C-5), 144.2 (C-4), 143.8 (C, C-4′), 138.8 (C, C-1′), 137.8 (C, C-8), 127.8 (C, C-1), 126.9 (C, C-2), 121.1 (CH, C-7), 120.4 (CH, C-6′), 113.9 (CH, C-5′), 112.1 (CH, C-3), 111.7 (CH, C-6), 110.1 (CH, C-2′), 55.9 (CH_3, OMe), 55.8 (CH_3, OMe), 51.1 (CH, C-7′), 42.1 (C-8′), 22.2 (CH_3, H_3-9), 18.8 (CH_3, H_3-9′); HRESIMS *m/z* 327.1597 $[M + H]^+$ (calcd for $C_{20}H_{23}O_4$, 327.1591).

(−)-4′-*O*-Demethylcyclogalgravin (12): white, amorphous powder; $[\alpha]^{25}_D$ −108.6 (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (1H, d, J = 8.4 Hz, H-5′), 6.60 (1H, s, H-6), 6.56 (1H, d, J = 2 Hz, H-2′), 6.54 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.53 (1H, s, H-3), 6.12 (1H, d, J = 1.2 Hz, H-7), 5.40 (1H, s, D_2O exchangeable, OH-4′), 3.86 (3H, s, OMe), 3.76 (3H, s, OMe), 3.75 (3H, s, OMe), 3.64 (1H, d, J = 2.8 Hz, H-7′), 2.35 (1H, m, H-8′), 1.77 (3H, s, H_3-9), 1.05 (1H, d, J = 7.2 Hz, H_3-9′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6 (C, C-5), 147.5 (C, C-4), 146.2 (C, C-3′), 143.8 (C, C-4′), 138.8 (C, C-1′), 137.6 (C, C-8), 127.4 (C, C-2), 127.1 (C, C-1), 121.1 (CH, C-7), 120.4 (CH, C-6′), 113.9 (CH, C-5′), 112.9 (CH, C-3), 110.1 (CH, C-2′), 108.9 (CH, C-6), 55.9 (CH_3, 2 × OMe), 55.8 (CH_3, OMe), 50.9 (CH, C-7′), 42.2 (CH, C-8′), 22.2 (CH_3, C-9), 18.7 (CH_3, C-9′); HRESIMS *m/z* $[M + H]^+$ 341.1751 (calcd for $C_{21}H_{25}O_4$, 341.1747).

Microbial Transformation of (−)-Isogalbulin (2) by *B. bassiana* ATCC 7159. Screening-scale microbial transformation of **2** by *B. bassiana* ATCC 7159 was carried out in a 125 mL Erlenmeyer flask containing 25 mL of potato dextrose broth (PDB, Difco, Plymouth, MN). The flask was placed on a rotary shaker operating at 220 rpm at 28 °C. Substrate (**2**, 2.5 mg in 0.25 mL of DMF) was added to 24 h old second-stage culture broth. Culture control consisted of fermentation broth of *B. bassiana* ATCC 7159 without the substrate but with the same volume of DMF, and the substrate control consisted of sterile PDB medium with the same amount of a solution of **2** in DMF. Both controls were incubated under the same conditions. Formation of microbial transformation metabolites was followed by TLC. Preparative-scale fermentation was performed under the same conditions as small-scale fermentations in 2 × 250 mL Erlenmeyer flasks, each holding

50 mL of the culture broth. A total of 10 mg of **2** was used. After 20 days of incubation, the cultures were filtered; the combined filtrate was neutralized with 1 M NaOH and extracted with EtOAc (3 × 200 mL). The combined EtOAc layer was washed with H_2O (200 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give EtOAc extract (8.3 mg) as a yellow syrup. This extract was then separated by preparative TLC [eluant: hexanes/EtOAc/$CHCl_3$ (6:3:1)] to yield (−)-8-hydroxyisogalbulin (**6**) (0.7 mg, R_f 0.6), identical (^{1}H NMR and LRMS) with previously obtained sample.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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