Biotransformation of *ent*-Atisenes and *ent*-Beyerenes by *Rhizopus nigricans* and *Fusarium moniliforme* Cultures

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Microbial transformations of *ent*-atisene and *ent*-beyerene compounds were carried out using cultures of *R. nigricans* and *F. moniliforme*. *Rhizopus nigricans* metabolized all of the substrates, at higher yields than *F. moniliforme*, to give an *ent*-3β hydroxylation (60−65% for *ent*-atisenes and 80−85% for *ent*-beyerenes). Moreover, the double bond of the *ent*-beyerene skeleton was epoxidized by both fungi at lower yields (3−6%). *Fusarium moniliforme* produced a number of additional minor products by hydroxylation at other positions.

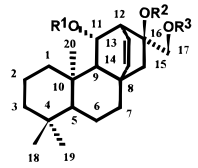
Numerous papers have been published on the microbial transformations of diterpene compounds,[1] although those devoted to the biotransformation of *ent*-beyerene[2−7] and *ent*-atisene[8,9] diterpenes are scarce. In previous papers we reported the bioconversion of some *ent*-beyerenes by *Rhizopus nigricans* and *Curvularia lunata*.[4,7] The biotransformation of the 14β-hydroxylbeyerene derivative[4] by *R. nigricans* gave the *ent*-15α,16α-epoxy derivative as the main metabolite in limited yield (4%). When the substrates were *ent*-beyerenes with a ketone function,[7] the main action of *R. nigricans* was hydroxylation at C-3, although hydroxylations at C-1, C-12, and C-19 were also observed.

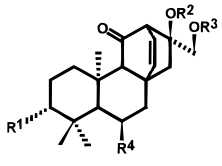
We describe herein the results of the biotransformation of *ent*-beyer-15-ene derivatives with different functions at C-12 by *R, nigricans* and *F. moniliforme*. In addition, we report the microbial transformations of various *ent*-atisene compounds functionalized at C-11, C-16, and C-17 by the mentioned fungi.

Results and Discussion

Acetylation of *ent*-11β,16α,17-trihydroxyatis-13-ene (atisideritol, **1**)[10,11] at room temperature gave principally compound **2**, the C-11 and C-17 diacetate, and triacetyl derivative **3**. When this acetylation was carried out at 0 °C and for a shorter time, however, acetate **4** was obtained. Mild saponification of *ent*-11β,17-diacetoxy-16α-hydroxyatis-13-ene (**2**) gave substrate **5**, which was the *ent*-11β monoacetylated derivative. Oxidation of **4** yielded *ent*-17-acetoxy-16α-hydroxyatis-13-en-11-one (**6**), a new substrate for the following biotransformations. On the other hand, saponification of *ent*-7α,12α,17-triacetoxybeyer-15-ene (**7**)[12] gave a monoacetylated compound (**8**). The C-17 hydroxyl group was silylated to give the 17-(*tert*-butyldimethylsilyl) derivative (**9**). Oxidation of **9** with CrO_3−Py under mild conditions gave the 12-*oxo* intermediate, compound **10**, and reaction of **10** with BF_3−CH_2Cl_2 yielded *ent*-7α-acetoxy-17-hydroxybeyer-15-en-12-one (**11**). Both *ent*-beyerenes **8** and **11** were then used as substrates for the microbial transformations.

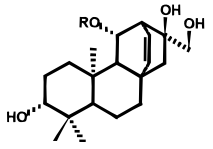
Incubation of **5** with *R. nigricans* for 8 days gave metabolites **12** and **13** and a small amount of recovered **5**. Metabolite **12** contained an additional oxygen, as was deduced from its molecular ion peak (*m*/*z* 378). Its ^{1}H-NMR spectrum showed a signal at δ 3.22 (1H, dd, J_1 = 4.5 Hz, J_2 = 11.7 Hz) assigned to H-3, and detailed study of the ^{13}C-NMR data indicated that the new hydroxylation was equatorial at C-3. Thus, an α-effect on C-3 ($\Delta\delta$ +38.1), β-effects on C-2 ($\Delta\delta$ +8.5) and C-4 ($\Delta\delta$ +5.3), and γ-effects on C-1 ($\Delta\delta$ −1.5), C-18 ($\Delta\delta$ −5.4), and C-19 ($\Delta\delta$ −4.9) were observed. Metabolite **13** was the result of equatorial hydroxylation of substrate **5** at C-3 and deacetylation at C-11. The structure of **13** was supported by analysis of the ^{13}C NMR. Thus, the primary action of *R. nigricans* on substrate **5** was *ent*-3β hydroxylation. Biotransformation of substrate **5** by *F. moniliforme* for 12 days gave only the minor metabolite **14**. Metabolite **14** had a new hydroxyl group whose geminal proton resonated at δ 3.49 (1H, dd, J_1 = 5.1 Hz, J_2 = 11.5 Hz). Comparison of the ^{13}C-NMR spectra of **5** and metabolite **14** indicated that *ent*-7α hydroxylation had occurred. ^{13}C-NMR signals were observed for C-7 (α-effect, $\Delta\delta$ +39.1), C-16 and C-6 (β-effects, $\Delta\delta$ +9.5 and $\Delta\delta$ +4.4), and C-14 and C-15 (γ-effects, $\Delta\delta$ −5.1 and $\Delta\delta$ −3.7).

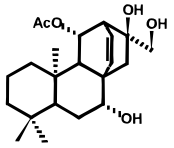




1 R^1=R^2=R^3=H
2 R^1=R^3=Ac, R^2=H
3 R^1=R^2=R^3=Ac
4 R^1=R^2=H, R^3=Ac
5 R^1=Ac, R^2=R^3=H

6 R^1=R^2=R^4=H, R^3=Ac
15 R^1=R^2=R^3=R^4=H
16 R^1=R^2=R^3=H, R^4=OH
17 R^1=OH, R^2=R^4=H, R^3=Ac
18 R^1=OH, R^2=R^3=R^4=H





12 R=Ac
13 R=H

14

Incubation of **6** by *F. moniliforme* for 12 days gave metabolites **15** and **16**. Metabolite **15** was the starting material deacetylated at C-17, and metabolite **16** was a trihydroxylated *ent*-atisenone with a molecular weight

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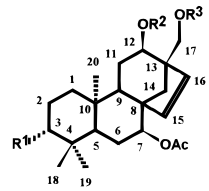
of 334. The ^{1}H-NMR spectrum of **16** showed a signal at δ 4.04 (1H, ddd, $J_1 = J_2 = 10.5$ Hz, $J_3 = 4.5$ Hz) due to an axial proton. The multiplicity and coupling constants of this signal indicated that the new hydroxyl group was situated at C-6 with an *ent*-6α configuration, and ^{13}C-NMR effects observed corroborated this conclusion.

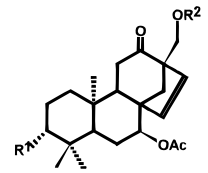
Biotransformation of substrate **6** with *R. nigricans* provided four metabolites: **13**, **15**, **17**, and **18**. (Metabolite **13** was also obtained in the biotransformation of **5** by *R. nigricans*.) Metabolite **15** in this biotransformation was derived via deacetylation of **6** at C-17 with *F. moniliforme*. Another metabolite (**17**) obtained from this incubation showed a molecular peak of *m/z* 378, consistent with an additional hydroxyl group. The ^{1}H-NMR signal of the proton geminal to the new hydroxyl group at δ 3.21 (1H, dd, $J_1 = 4.6$ Hz, $J_2 = 11.5$ Hz) indicated an *ent*-3β-H configuration. Metabolite **18** had a similar signal in its ^{1}H-NMR spectrum; its hydroxymethylene group was deacetylated. Thus, *R. nigricans* yields derivatives hydroxylated primarily at C-3 of the *ent*-atisene skeleton.

Incubation of *ent*-beyerene **8** with *R. nigricans* for 12 days gave metabolites **19** and **20** and starting material. The structure of metabolite **19**, formed as the result of the hydroxylation of substrate **8**, was deduced from its spectroscopic properties. The proton signal geminal to the new hydroxyl appeared in the ^{1}H-NMR spectrum at δ 3.18 (1H, dd, $J_1 = J_2 = 7.0$ Hz). From the multiplicity and coupling constants of this signal, it was deduced that the new hydroxyl group must be situated at C-3 in an *ent*-3β configuration. This conclusion was supported by the ^{13}C-NMR data, inasmuch as ^{13}C-NMR effects similar to the above were observed for C-1, C-2, C-3, C-4, C-18, and C-19. Metabolite **20** did not present signals assignable to a double bond in its ^{1}H-NMR spectrum, but the spectrum contained signals consistent with an epoxide group and a new hydroxyl group. The new hydroxyl was at C-3 in an *ent*-3β orientation, since its geminal proton was located at δ 3.18 (1H, dd, $J_1 = 6.2$ Hz, $J_2 = 8.4$ Hz) as in **19**. Thus, *R. nigricans* highly functionalized substrate **8** by equatorial (*S*) hydroxylation at C-3 and, at a slower rate, epoxidized the 15/16 double bond. When substrate **8** was incubated with *F. moniliforme* for 17 days, only a minor metabolite (**21**) and unaltered substrate were isolated. Metabolite **21** also had no signals typical of the 15/16 double bond in its ^{1}H-NMR spectrum. There were, however, signals at δ 3.37 (1H, d, $J = 3.1$ Hz) and at δ 3.14 (1H, d, $J = 3.1$ Hz) assigned to both protons geminal to an epoxide group between C-15 and C-16. The structure of metabolite **21** was also confirmed by chemical correlation. Thus, treatment of **8** with *m*-CPBA gave rise to a product identical to metabolite **21**.

Biotransformation of another *ent*-beyerene substrate (**11**) by *R. nigricans* for 12 days yielded metabolites **22** and **23** and starting material. The *ent*-3α-H axial signal of **23** was located at δ 3.18 (1H, dd, $J_1 = 4.7$ Hz, $J_2 = 11.4$ Hz), and the expected ^{13}C-NMR effects were observed for the ring A carbons. Compound **23** had spectroscopic properties similar to those of **20**. Again, the primary action of *R. nigricans* on the *ent*-beyerene skeleton was an *ent*-3β hydroxylation independent of the function at C-12. When the same substrate (**11**) was biotransformed by *F. moniliforme* for 17 days, three minor metabolites were obtained: **24**, **25**, and **26**, along with unaltered **4**. In accordance with previous results and with ^{1}H- and ^{13}C-NMR spectra of **24**, we established that this compound had an *ent*-15α,16α-epoxy group. The spectroscopic properties of **25** indicated that it was an *ent*-beyerene compound with a new hydroxyl group at C-1 in an *ent*-1β configuration. Its geminal axial proton appeared at δ 3.35 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 10.5$ Hz) in its ^{1}H-NMR spectrum. This conclusion was confirmed by the ^{13}C-NMR effects observed: α-effect on C-1 ($\Delta\delta$ +41.2), β-effects on C-2 ($\Delta\delta$ +8.3) and on C-10 ($\Delta\delta$ +5.5), and γ-effects on C-3 ($\Delta\delta$ −2.0), on C-9 ($\Delta\delta$ −2.7), and on C-20 ($\Delta\delta$ −4.3). Metabolite **26** had the same function as **25** at C-1, and its C-7 hydroxyl group had been deacetylated. The structure of **24** was also corroborated by chemical correlation, since treatment of the *ent*-beyerene compound **11** with *m*-CPBA gave a product identical to **24**. Another product appeared in this epoxidation (**27**) as the result of a Baeyer−Villiger process. Thus, we conclude that the primary action of *R. nigricans* on *ent*-atisene and *ent*-beyerene compounds, having the C-ring highly functionalized, is *ent*-3β hydroxylation.



7 R^1=H, R^2=R^3=Ac
8 R^1= R^2=R^3=H
9 R^1=R^2=H, R^3=SMDBT
19 R^1=OH, R^2=R^3=H



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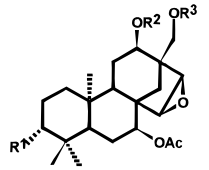
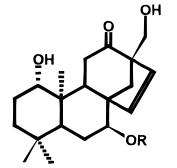
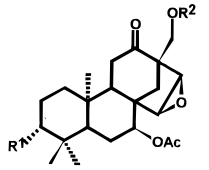
11 R^1= R^2=H
22 R^1=OH, R^2=H



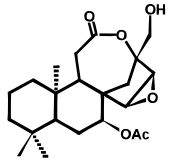
20 R^1=OH, R^2=R^3=H
21 R^1= R^2=R^3=H



25 R=Ac
26 R=H



23 R^1=OH, R^2=H
24 R^1= R^2=H



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Experimental Section

General Experimental Procedures. Melting points were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ or CH_3OH (1-dm tube) with a Jasco DIP-370 polarimeter. IR spectra were recorded on a Perkin-Elmer 983 G spectrometer or on a Nicolet 20SX FT-IR spectrometer. MS were determined with CI (CH_4) in a Hewlett-Packard 5988 A spectrometer. HRMS were obtained with a VG AutoSpec-Q high-resolution spectrometer. NMR spectra (400.13 MHz ^{1}H, 300.13 MHz ^{1}H, and 100.62 MHz ^{13}C) were performed in $CDCl_3$ or

MeOD (which also provided the lock signal) in Brucker ARX-400 and Brucker AM-300 spectrometers. The assignments of ^{13}C chemical shifts were done with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Si gel SDS 60 A column chromatography (40−60 μm) was used for flash chromatography. CH_2Cl_2 or $CHCl_3$ containing increasing amounts of Me_2CO was used as the eluent. Analytical plates (Si gel, Merck 60 G) were used for TLC analysis, and spots were visualized by spraying with H_2SO_4−AcOH, followed by heating to 120°. Starting materials for this work, *ent*-11β,16α,17-trihydroxyatis-13-ene (**1**) and *ent*-7α,12α,17-triacetoxybeyer-15-ene (**7**), were isolated from *Sideritis granatensis* using published procedures.[10−12]

Organism, Media, and Culture Conditions. *Rhizopus nigricans* CECT 2072 and *Fusarium moniliforme* CECT 2152 were obtained from the Colección Española de Cultivos Tipo (CECT), Departamento de Microbiología, Universidad de Valencia, Spain. The fungal cultures were stored in YEPGA medium containing 1% yeast extract, 1% peptone, 2% glucose, and 2% agar, at pH 5. A medium composed of peptone (0.1%), yeast extract (0.1%), beef extract (0.1%), and glucose (0.5%) at pH 5.7 in H_2O was used in all transformation experiments. Erlenmeyer flasks (250 mL) containing 80 mL of medium were inoculated with a suspension of *R. nigricans* or *F. moniliforme*. Incubations were maintained at 28 °C with gyratory shaking (150 rpm) for 6 days, after which the substrate (**5, 6**, **8**, or **11**) in EtOH was added.

Recovery and Purification of Metabolites. Cultures were filtered and pooled, cells were washed with H_2O and the liquid was saturated with NaCl and extracted with CH_2Cl_2. These extracts were mixed, dried over anhydrous Na_2SO_4, and evaporated at 40 °C under reduced pressure. Mixtures of products obtained were chromatographed on Si columns.

Acetylation of *ent*-11β,16α,17-Trihydroxyatis-13-ene (1). Compound **1** (900 mg) was acetylated with Ac_2O−Py (2:4 mL) for 1 h at room temperature. Purification by flash chromatography provided diacetate **2** (720 mg, 80%) and triacetate **3** (90 mg, 10%).

***ent*-11β,17-Diacetoxy-16α-hydroxyatis-13-ene (2):** syrup; $[\alpha]^{25}_D$ −73° (*c* 1, $CHCl_3$); IR νmax ($CHCl_3$) 3500, 1720, 1660, 750; ^1H NMR (δ, $CDCl_3$) 6.16 (1H, d, J = 8.2 Hz, H-14), 5.89 (1H, dd, J_1 = 6.1 Hz, J_2 = 8.2 Hz, H-15), 5.86 (1H, dd, J_1 = 3.0 Hz, J_2 = 12.0 Hz, H-11), 3.82 (1H, d, J = 11.2 Hz, H-17), 3.67 (1H, d, J = 11.2 Hz, H-17), 2.77 (1H, dd, J_1 = 3.0 Hz, J_2 = 6.1 Hz, H-12), 2.06 (3H, s, MeCOO), 1.96 (3H, s, MeCOO), 0.93, 0.83, 0.80 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 40.6 (C-1), 18.3 (C-2), 41.9 (C-3), 33.2 (C-4), 56.0 (C-5), 19.5 (C-6), 36.5 (C-7), 39.9 (C-8), 58.9 (C-9), 38.8 (C-10), 73.0 (C-11), 44.9 (C-12), 127.5 (C-13), 139.8 (C-14), 48.3 (C-15), 74.2 (C-16), 70.3 (C-17), 34.0 (C-18), 22.1 (C-19), 17.2 (C-20), 21.8 (*Me*CO), 21.0 (*Me*CO), 171.0 (Me*CO*), 170.6 (Me*CO*); CIMS (CH_4) *m*/*z* 405 $[M + 1]^+$ (10), 387 (25), 345 (25), 285 (100); HRMS (CI) calcd for $C_{24}H_{36}O_5$ 405.2480, found 405.2475.

***ent*-11β,16α,17-Triacetoxyatis-13-ene (3):** syrup; $[\alpha]^{25}_D$ −16° (*c* 1, $CHCl_3$); IR νmax ($CHCl_3$) 1740, 1645, 740; ^1H NMR (δ, $CDCl_3$) 6.22 (1H, d, J = 8.0 Hz, H-14), 5.90 (1H, dd, J_1 = 6.4 Hz, J_2 = 8.0 Hz, H-15), 5.82 (1H, dd, J_1 = 2.8 Hz, J_2 = 8.9 Hz, H-11), 4.51 (1H, d, J = 12.0 Hz, H-17), 3.93 (1H, d, J = 12.0 Hz, H-17), 3.07 (1H, dd, J_1 = 2.8 Hz, J_2 = 6.4 Hz, H-12), 2.05 (3H, s, MeCOO), 2.04 (3H, s, MeCOO), 1.98 (3H, s, MeCOO), 0.93, 0.84, 0.81 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 40.6 (C-1), 18.3 (C-2), 41.9 (C-3), 33.3 (C-4), 56.1 (C-5), 19.5 (C-6), 36.3 (C-7), 39.8 (C-8), 59.7 (C-9), 38.9 (C-10), 71.6 (C-11), 43.0 (C-12), 126.3 (C-13), 140.4 (C-14), 46.6 (C-15), 83.1 (C-16), 65.4 (C-17), 34.0 (C-18), 22.0 (C-19), 17.3 (C-20), 22.2 (*Me*CO), 21.8 (*Me*CO), 20.9 (*Me*CO), 170.8 (Me*CO*), 170.7 (Me*CO*), 170.3 (Me*CO*); CIMS (CH_4) *m*/*z* 447 $[M + 1]^+$ (8), 387 (4), 327 (20), 267 (100); HRMS (CI) calcd for $C_{26}H_{38}O_6$ 447.2746, found 447.2725.

Partial Acetylation of *ent*-11β,16α,17-Trihydroxyatis-13-ene (1). Compound **1** (800 mg) was acetylated with Ac_2O−Py (2:4 mL) for 1 h at 0° C. Purification by flash chromatography provided **4** (720 mg, 90%).

***ent*-17-Acetoxy-11β,16α-dihydroxyatis-13-ene (4):** mp 147−149 °C; $[\alpha]^{25}_D$ −25° (*c* 0.5, $CHCl_3$); IR νmax ($CHCl_3$) 3480, 1735, 1650, 750; ^1H NMR (δ, $CDCl_3$) 6.30 (1H, d, J = 8.0 Hz, H-14), 6.00 (1H, dd, J_1 = 6.0 Hz, J_2 = 8.0 Hz, H-15), 4.75 (1H, br d, J = 8.0 Hz, H-11), 3.85 (1H, d, J = 11.2 Hz, H-17), 3.73 (1H, d, J = 11.2 Hz, H-17), 2.83 (1H, dd, J_1 = 3.1 Hz, J_2 = 6.0 Hz, H-12), 2.10 (3H, s, MeCOO), 0.97, 0.86, 0.84 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 41.6 (C-1), 19.8 (C-2), 42.0 (C-3), 33.2 (C-4), 56.0 (C-5), 18.5 (C-6), 36.7 (C-7), 40.2 (C-8), 60.2 (C-9), 39.0 (C-10), 71.5 (C-11), 48.5 (C-12), 127.3 (C-13), 142.8 (C-14), 49.1 (C-15), 74.5 (C-16), 70.8 (C-17), 34.2 (C-18), 22.3 (C-19), 18.0 (C-20), 21.0 (*Me*CO), 171.4 (Me*CO*); CIMS (CH_4) *m*/*z* 363 $[M + 1]^+$ (12), 303 (22), 285 (60), 267 (100); HRMS (CI) calcd for $C_{22}H_{34}O_4$ 363.2535, found 363.2549.

Saponification of *ent*-11β,17-Diacetoxy-16α-hydroxyatis-13-ene (2). Compound **2** (700 mg) was dissolved in 25 mL of a MeOH−H_2O (30:70) with 5% of KOH. The reaction was maintained at room temperature for 5 h, after which time it was neutralized, extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4, and purified by flash chromatography, yielding 630 mg of **5** (90%).

***ent*-11β-Acetoxy-16α,17-dihydroxyatis-13-ene (5):** mp 144−146 °C; $[\alpha]^{25}_D$ −62° (*c* 1, $CHCl_3$); IR νmax ($CHCl_3$) 3450, 1730, 1655, 745; ^1H NMR (δ, $CDCl_3$) 6.15 (1H, d, J = 8.0 Hz, H-14), 5.91 (1H, dd, J_1 = 6.1 Hz, J_2 = 8.0 Hz, H-15), 5.78 (1H, dd, J_1 = 2.8 Hz, J_2 = 8.8 Hz, H-11), 3.29 (1H, d, J = 11.1 Hz, H-17), 3.21 (1H, d, J = 11.1 Hz, H-17), 2.90 (1H, dd, J_1 = 2.8 Hz, J_2 = 6.1 Hz, H-12), 1.97 (3H, s, MeCOO), 0.94, 0.84, 0.81 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 40.1 (C-1), 18.5 (C-2), 40.8 (C-3), 33.4 (C-4), 56.1 (C-5), 19.7 (C-6), 36.8 (C-7), 42.0 (C-8), 59.0 (C-9), 40.8 (C-10), 74.3 (C-11), 44.1 (C-12), 127.7 (C-13), 139.9 (C-14), 48.5 (C-15), 75.7 (C-16), 69.3 (C-17), 34.2 (C-18), 22.2 (C-19), 17.4 (C-20), 22.0 (*Me*CO), 171.4 (Me*CO*); CIMS (CH_4) *m*/*z* 363 $[M + 1]^+$ (5), 303 (12), 285 (45), 267 (100); HRMS (CI) calcd for $C_{22}H_{34}O_4$ 363.2535, found 363.2506.

Oxidation of *ent*-17-Acetoxy-11β,16α-dihydroxyatis-13-ene (4). Compound **4** (700 mg) was dissolved in Me_2CO (12 mL) and oxidized with Jones' reagent for 1 h at room temperature. Purification by flash chromatography gave 625 mg of **6** (90%).

***ent*-17-Acetoxy-16α-hydroxyatis-13-en-11-one (6):** mp 128−130 °C; $[\alpha]^{25}_D$ −142° (*c* 0.5, $CHCl_3$); IR νmax ($CHCl_3$) 3505, 1718, 1730, 740; ^1H NMR (δ, $CDCl_3$) 6.21

(1H, d, J = 7.9 Hz, H-14), 5.89 (1H, dd, J_1 = 6.2 Hz, J_2 = 7.9 Hz, H-15), 3.88 (1H, d, J = 11.3 Hz, H-17), 3.80 (1H, d, J = 11.3 Hz, H-17), 3.14 (1H, d, J = 6.2 Hz, H-12), 2.59 (1H, ddd, J_1 = J_2 = 3.3 Hz, J_3 = 13.4 Hz, H-1), 2.08 (3H, s, MeCOO), 1.89 (1H, ddd, J_1 = J_2 = 3.0 Hz, J_3 = 13.1 Hz, H-7), 1.79 (1H, s, H-9), 0.86, 0.77, 0.69 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 39.6 (C-1), 18.5 (C-2), 41.9 (C-3), 33.4 (C-4), 55.3 (C-5), 18.8 (C-6), 36.7 (C-7), 39.8 (C-8), 59.0 (C-9), 41.6 (C-10), 210.2 (C-11), 60.9 (C-12), 122.7 (C-13), 142.1 (C-14), 47.4 (C-15), 75.5 (C-16), 68.8 (C-17), 33.8 (C-18), 21.8 (C-19), 17.4 (C-20), 20.9 (*Me*CO), 170.9 (Me*CO*); CIMS (CH_4) m/z 361 [M + 1]$^+$ (8), 241 (15), 223 (30), 205 (100); HRMS (CI) calcd for $C_{22}H_{32}O_4$ 361.2378, found 361.2371.

Saponification of *ent*-7α,12α,17-Triacetoxybeyer-15-ene (7). Product **7** (1700 mg) was saponified with 70 mL of a MeOH−H_2O (30:70) solution with 5% of KOH for 5 h at room temperature. Working as described above and after flash chromatography, compound **8** was obtained (1530 mg, 90%).

***ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (8):** mp 165−167 °C; $[\alpha]^{25}_D$ −1° (c 0.5, $CHCl_3$); IR νmax ($CHCl_3$) 3450, 3055, 1730, 1255, 770; ^{1}H NMR (δ, $CDCl_3$) 5.70 (1H, d, J = 5.8 Hz, H-16), 5.56 (1H, d, J = 5.8 Hz, H-15), 4.91 (1H, dd, J_1 = J_2 = 2.8 Hz, H-7), 3.97 (1H, br s, H-12), 3.79 (1H, d, J = 10.6 Hz, H-17), 3.57 (1H, d, J = 10.6 Hz, H-17), 2.04 (3H, s, MeCOO), 0.78, 0.78, 0.67 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 38.6 (C-1), 18.3 (C-2), 41.8 (C-3), 32.5 (C-4), 47.8 (C-5), 25.2 (C-6), 75.6 (C-7), 53.4 (C-8), 44.2 (C-9), 36.8 (C-10), 29.2 (C-11), 70.1 (C-12), 53.1 (C-13), 44.8 (C-14), 136.0 (C-15), 133.4 (C-16), 68.9 (C-17), 33.2 (C-18), 21.6 (C-19), 13.8 (C-20), 21.3 (*Me*CO), 170.8 (Me*CO*); CIMS (CH_4) m/z 363 [M + 1]$^+$ (10), 303 (12), 285 (24), 267 (100); HRMS (CI) calcd for $C_{22}H_{34}O_4$ 363.2535, found 363.02547.

Silylation at C-17 of *ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (8). Compound **8** (900 mg) was dissolved in 10 mL of pyridine and TBDMS (590 mg) was added. The reaction was maintained at room temperature for 24 h, and it was stopped by addition of a few drops of MeOH. The reaction mixture was extracted with CH_2Cl_2 and dried with anhydrous Na_2SO_4. After flash chromatography, 830 mg of **9** (90%) was obtained.

***ent*-7α-Acetoxy-12α-hydroxy-17-(*tert*-butyldimethylsilyl)beyer-15-ene (9):** syrup; $[\alpha]^{25}_D$ 34° (c 1, $CHCl_3$); IR νmax ($CHCl_3$) 3475, 1728, 1265, 1245; ^{1}H NMR (δ, $CDCl_3$) 5.72 (1H, d, J = 5.7 Hz, H-16), 5.48 (1H, d, J = 5.7 Hz, H-15), 4.94 (1H, dd, J_1 = J_2 = 2.8 Hz, H-7), 3.98 (1H, br s, H-12), 3.88 (1H, d, J = 10.5 Hz, H-17), 3.54 (1H, d, J = 10.5 Hz, H-17), 2.34 (1H, dd, J_1 = 10.6 Hz, J_2 = 16.8 Hz, H-11), 2.24 (1H, dd, J_1 = 6.6 Hz, J_2 = 16.8 Hz, H-11), 2.04 (3H, s, MeCOO), 0.87 (9H, s, Me_3C), 0.77, 0.77, 0.68 (3H each, s), 0.00 (6H, s, Me_2Si); ^{13}C NMR (δ, $CDCl_3$) 38.6 (C-1), 18.5 (C-2), 42.0 (C-3), 32.6 (C-4), 48.0 (C-5), 25.3 (C-6), 75.6 (C-7), 53.4 (C-8), 44.1 (C-9), 36.9 (C-10), 28.7 (C-11), 70.5 (C-12), 53.5 (C-13), 45.6 (C-14), 136.1 (C-15), 133.2 (C-16), 70.4 (C-17), 33.3 (C-18), 21.7 (C-19), 14.0 (C-20), 25.8 (*Me*$_3$C), 18.2 (Me$_3$*C*), −5.6 (*Me*$_2$Si), 21.3 (*Me*CO), 170.7 (Me*CO*); CIMS (CH_4) m/z 477 [M + 1]$^+$ (5), 417 (18), 399 (4), 267 (100); HRMS (CI) calcd for $C_{28}H_{48}O_4$ 477.3400, found 477.3387.

Oxidation of *ent*-7α-Acetoxy-12α-hydroxy-17-(*tert*-butyldimethylsilyl)beyer-15-ene (9). CrO_3 (4200 mg) was added to a stirred solution of 800 mg of **9** in pyridine (10 mL). After 2 h at room temperature, the reaction mixture was extracted with Et_2O, washed with saturated $KHSO_4$, dried, and evaporated. Purification by flash chromatography provided 730 mg of **10** (90%).

***ent*-7α-Acetoxy-17-(*tert*-butyldimethylsilyl)beyer-15-en-12-one (10):** mp 148−150 °C; $[\alpha]^{25}_D$ −15° (c 0.5, $CHCl_3$); IR νmax ($CHCl_3$) 1748, 1732, 1245, 770; ^{1}H NMR (δ, $CDCl_3$) 5.95 (1H, d, J = 5.7 Hz, H-16), 5.78 (1H, d, J = 5.7 Hz, H-15), 5.07 (1H, dd, J_1 = J_2 = 2.8 Hz, H-7), 3.84 (1H, d, J = 10.5 Hz, H-17), 3.63 (1H, d, J = 10.5 Hz, H-17), 2.36 (1H, dd, J_1 = 10.5 Hz, J_2 = 16.8 Hz, H-11), 2.25 (1H, dd, J_1 = 6.7 Hz, J_2 = 16.8 Hz, H-11), 2.05 (3H, s, MeCOO), 0.84 (9H, s, Me_3C), 0.80, 0.80, 0.76 (3H each, s), 0.00 (6H, s, Me_2Si); ^{13}C NMR (δ, $CDCl_3$) 38.1 (C-1), 18.3 (C-2), 41.8 (C-3), 32.6 (C-4), 47.7 (C-5), 25.0 (C-6), 74.9 (C-7), 52.5 (C-8), 49.8 (C-9), 37.6 (C-10), 36.3 (C-11), 210.4 (C-12), 63.0 (C-13), 49.0 (C-14), 137.5 (C-15), 134.7 (C-16), 61.7 (C-17), 33.1 (C-18), 21.5 (C-19), 13.5 (C-20), 26.0 (*Me*$_3$C), 18.4 (Me$_3$*C*), −5.4 (*Me*$_2$Si), 21.3 (*Me*CO), 170.4 (Me*CO*); CIMS (CH_4) m/z 475 [M + 1]$^+$ (3), 415 (12), 397 (6), 265 (100); HRMS (CI) calcd for $C_{28}H_{46}O_4$ 475.3243, found 475.3232.

Cleavage of *ent*-7α-Acetoxy-17-(*tert*-butyldimethylsilyl)beyer-15-en-12-one (10). A stirred solution of 700 mg of **10** in CH_2Cl_2 (20 mL) was treated with a few drops of BF_3 at room temperature for 3 h. The solution was concentrated, and after purification and separation by flash chromatography, **11** (625 mg, 90%) was obtained.

***ent*-7α-Acetoxy-17-hydroxybeyer-15-en-12-one (11):** mp 154−156 °C; $[\alpha]^{25}_D$ −299° (c 0.5, $CHCl_3$); IR νmax ($CHCl_3$) 3505, 1735, 1728, 1260; ^{1}H NMR (δ, $CDCl_3$) 6.04 (1H, d, J = 5.7 Hz, H-16), 5.70 (1H, d, J = 5.7 Hz, H-15), 5.09 (1H, dd, J_1 = J_2 = 2.8 Hz, H-7), 3.70 (1H, dd, J_1 = 5.8 Hz, J_2 = 11.7 Hz, H-17), 3.60 (1H, dd, J_1 = 7.6 Hz, J_2 = 11.7 Hz, H-17), 2.39 (1H, dd, J_1 = 10.4 Hz, J_2 = 17.0 Hz, H-11), 2.29 (1H, dd, J_1 = 6.8 Hz, J_2 = 17.0 Hz, H-11), 2.05 (3H, s, MeCOO), 0.81, 0.81, 0.77 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 38.1 (C-1), 21.2 (C-2), 41.8 (C-3), 32.6 (C-4), 47.7 (C-5), 25.2 (C-6), 74.5 (C-7), 52.3 (C-8), 47.7 (C-9), 37.4 (C-10), 36.3 (C-11), 213.2 (C-12), 62.9 (C-13), 49.7 (C-14), 138.5 (C-15), 133.3 (C-16), 64.0 (C-17), 33.1 (C-18), 21.5 (C-19), 13.5 (C-20), 21.2 (*Me*CO), 170.3 (Me*CO*); CIMS (CH_4) m/z 361 [M + 1]$^+$ (1), 301 (100), 241 (89), 229 (93); HRMS (CI) calcd for $C_{22}H_{32}O_4$ 361.2378, found 361.2363.

Biotransformation of *ent*-11β-Acetoxy-16α,17-dihydroxyatis-13-ene (5) with *Rhizopus nigricans*. Substrate **5** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 8 days, and the metabolites were recovered and chromatographed on Si to obtain **12** (94 mg, 30%), **13** (97 mg, 35%), and starting material (60 mg, 20%).

***ent*-11β-Acetoxy-3β,16α,17-trihydroxyatis-13-ene (12):** mp 230−232 °C; $[\alpha]^{25}_D$ −81° (c 0.5, CH_3OH); IR νmax ($CHCl_3$) 3480, 2950, 1718, 1174, 740; ^{1}H NMR (δ, $CDCl_3$) 6.15 (1H, d, J = 8.0 Hz, H-14), 5.93 (1H, dd, J_1 = 6.1 Hz, J_2 = 8.0 Hz, H-15), 5.83 (1H, dd, J_1 = 2.8 Hz, J_2 = 8.8 Hz, H-11), 3.30 (1H, d, J = 11.0 Hz, H-17), 3.21 (1H, d, J = 11.0 Hz, H-17), 3.22 (1H, dd, J_1 = 4.5 Hz, J_2 = 11.7 Hz, H-3), 2.90 (1H, dd, J_1 = 2.8 Hz, J_2 = 6.4 Hz, H-12), 1.99 (3H, s, MeCOO), 0.97, 0.95, 0.80 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 38.6 (C-1), 27.0 (C-2), 78.8

(C-3), 38.7 (C-4), 55.5 (C-5), 19.4 (C-6), 36.6 (C-7), 39.1 (C-8), 58.9 (C-9), 39.9 (C-10), 73.8 (C-11), 44.2 (C-12), 127.8 (C-13), 139.6 (C-14), 48.2 (C-15), 75.6 (C-16), 69.2 (C-17), 28.7 (C-18), 17.3 (C-19), 15.6 (C-20), 21.9 (*Me*CO), 171.0 (Me*CO*); CIMS (CH_4) *m/z* 379 $[M + 1]^+$ (10), 361 (8), 343 (20), 325 (23), 319 (100); HRMS (CI) calcd for $C_{22}H_{34}O_5$ 379.2425, found 379.2440.

***ent*-3*β*,11*β*,16α,17-Tetrahydroxyatis-13-ene (13):** mp 236−238 °C; $[\alpha]^{25}_D$ −26° (*c* 1, CH_3OH); IR *ν*max ($CHCl_3$) 3515, 2955, 1180, 745; ^{1}H NMR (*δ*, MeOD) 6.13 (1H, d, *J* = 7.9 Hz, H-14), 6.00 (1H, dd, J_1 = 6.3 Hz, J_2 = 7.9 Hz, H-15), 4.77 (1H, dd, J_1 = 2.8 Hz, J_2 = 8.5 Hz, H-11), 3.25 (1H, d, *J* = 11.2 Hz, H-17), 3.11 (1H, d, *J* = 11.2 Hz, H-17), 3.18 (1H, dd, J_1 = 4.5 Hz, J_2 = 11.6 Hz, H-3), 2.94 (1H, dd, J_1 = 2.8 Hz, J_2 = 6.3 Hz, H-12), 2.34 (1H, ddd, $J_1 = J_2$ = 3.4 Hz, J_3 = 13.2 Hz, H-1), 1.86 (1H, ddd, $J_1 = J_2$ = 3.1 Hz, J_3 = 13.2 Hz, H-7), 1.00, 0.98, 0.80 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 40.2 (C-1), 27.8 (C-2), 79.9 (C-3), 40.1 (C-4), 57.1 (C-5), 20.6 (C-6), 38.1 (C-7), 40.1 (C-8), 61.9 (C-9), 41.1 (C-10), 71.6 (C-11), 49.3 (C-12), 129.8 (C-13), 140.6 (C-14), 49.7 (C-15), 76.9 (C-16), 70.1 (C-17), 29.4 (C-18), 18.1 (C-19), 16.6 (C-20); CIMS (CH_4) *m/z* 337 $[M + 1]^+$ (5), 319 (10), 301 (18), 283 (100); HRMS (CI) calcd for $C_{20}H_{32}O_4$ 337.2378, found 337.2386.

Biotransformation of *ent*-11*β*-Acetoxy-16α,17-dihydroxyatis-13-ene (5) with *Fusarium moniliforme*. Substrate **5** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 12 days, and the metabolites were recovered and chromatographed on Si to obtain **14** (22 mg, 7%) and starting material (230 mg, 80%).

***ent*-11*β*-Acetoxy-7α,16α,17-trihydroxyatis-13-ene (14):** syrup; $[\alpha]^{25}_D$ −99° (*c* 1, $CHCl_3$); IR *ν*max ($CHCl_3$) 3460, 1740, 1645, 740; ^{1}H NMR (*δ*, $CDCl_3$) 6.45 (1H, d, *J* = 8.2 Hz, H-14), 5.99 (1H, dd, J_1 = 6.2 Hz, J_2 = 8.2 Hz, H-15), 5.84 (1H, dd, J_1 = 2.7 Hz, J_2 = 8.4 Hz, H-11), 3.32 (1H, d, *J* = 11.0 Hz, H-17), 3.24 (1H, d, *J* = 11.0 Hz, H-17), 3.49 (1H, dd, J_1 = 5.1 Hz, J_2 = 11.5 Hz, H-7), 2.89 (1H, dd, J_1 = 2.7 Hz, J_2 = 6.2 Hz, H-12), 1.98 (3H, s, MeCOO), 0.95, 0.87, 0.83 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 40.3 (C-1), 18.3 (C-2), 41.6 (C-3), 33.2 (C-4), 52.9 (C-5), 29.2 (C-6), 75.9 (C-7), 46.4 (C-8), 58.3 (C-9), 38.9 (C-10), 73.5 (C-11), 44.5 (C-12), 127.7 (C-13), 134.8 (C-14), 44.8 (C-15), 75.6 (C-16), 69.3 (C-17), 34.0 (C-18), 22.1 (C-19), 17.3 (C-20), 21.9 (*Me*CO), 171.1 (Me*CO*); CIMS (CH_4) *m/z* 379 $[M + 1]^+$ (6), 361 (12), 343 (25), 325 (30), 319 (100); HRMS (CI) calcd for $C_{22}H_{34}O_5$ 379.2425, found 379.2435.

Biotransformation of *ent*-17-Acetoxy-16α-hydroxyatis-13-en-11-one (6) with *Fusarium moniliforme*. Substrate **6** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 12 days, and the metabolites were recovered and chromatographed on Si to obtain **15** (22 mg, 8%), **16** (8.5 mg, 3%), and starting material (180 mg, 60%).

***ent*-16α,17-Dihydroxyatis-13-en-11-one (15):** mp 208−210 °C; $[\alpha]^{25}_D$ −139° (*c* 1, $CHCl_3$); IR *ν*max ($CHCl_3$) 3500, 1720, 745; ^{1}H NMR (*δ*, $CDCl_3$) 6.19 (1H, d, *J* = 7.9 Hz, H-14), 5.91 (1H, dd, J_1 = 6.2 Hz, J_2 = 7.9 Hz, H-15), 3.67 (1H, d, *J* = 11.1 Hz, H-17), 3.32 (1H, d, *J* = 11.1 Hz, H-17), 3.25 (1H, d, *J* = 6.2 Hz, H-12), 2.60 (1H, ddd, J_1 = 3.1 Hz, J_2 = 5.0 Hz, J_3 = 13.5 Hz, H-1), 1.89 (1H, ddd, $J_1 = J_2$ = 3.0 Hz, J_3 = 10.0 Hz, H-7), 1.80 (1H, s, H-9), 0.87, 0.77, 0.70 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 39.9 (C-1), 18.5 (C-2), 41.9 (C-3), 33.4 (C-4), 55.3 (C-5), 18.9 (C-6), 36.8 (C-7), 39.7 (C-8), 58.5 (C-9), 41.7 (C-10), 211.6 (C-11), 61.3 (C-12), 122.9 (C-13), 141.9 (C-14), 47.5 (C-15), 77.0 (C-16), 67.8 (C-17), 33.9 (C-18), 21.8 (C-19), 17.5 (C-20); CIMS (CH_4) *m/z* 319 $[M + 1]^+$ (4), 301 (17), 283 (100); HRMS (CI) calcd for $C_{20}H_{30}O_5$ 319.2273, found 319.2277.

***ent*-6α,16α,17-Trihydroxyatis-13-en-11-one (16):** syrup; $[\alpha]^{25}_D$ −5° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3440, 1725, 1650, 740; ^{1}H NMR (*δ*, $CDCl_3$) 6.24 (1H, d, *J* = 7.2 Hz, H-14), 5.92 (1H, dd, J_1 = 6.4 Hz, J_2 = 7.2 Hz, H-15), 4.04 (1H, ddd, $J_1 = J_2$ = 10.5 Hz, J_3 = 4.5 Hz, H-6), 3.35 (2H, br s, H-17), 3.27 (1H, d, *J* = 6.4 Hz, H-12), 2.65 (1H, ddd, $J_1 = J_2$ = 3.3 Hz, J_3 = 13.6 Hz, H-1), 1.83 (1H, s, H-9), 1.16, 1.02, 0.77 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 39.5 (C-1), 18.4 (C-2), 43.9 (C-3), 33.8 (C-4), 58.8 (C-5), 68.0 (C-6), 47.7 (C-7), 41.7 (C-8), 60.4 (C-9), 41.6 (C-10), 209.1 (C-11), 60.2 (C-12), 123.1 (C-13), 141.7 (C-14), 47.5 (C-15), 76.4 (C-16), 67.6 (C-17), 37.2 (C-18), 22.5 (C-19), 18.6 (C-20); CIMS (CH_4) *m/z* 335 $[M + 1]^+$ (3), 317 (8), 299 (30), 281 (100); HRMS (CI) calcd for $C_{20}H_{30}O_4$ 335.2222, found 335.2210.

Biotransformation of *ent*-17-Acetoxy-16α-hydroxyatis-13-en-11-one (6) with *Rhizopus nigricans*. Substrate **6** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 8 days, and the metabolites were recovered and chromatographed on Si to obtain **13** (8.5 mg, 3%), **15** (22 mg, 8%), **17** (35 mg, 11%), **18** (145 mg, 52%), and starting material (45 mg, 15%).

***ent*-17-Acetoxy-3*β*,16α-dihydroxyatis-13-en-11-one (17):** mp 184−186 °C; $[\alpha]^{25}_D$ −148° (*c* 1, $CHCl_3$); IR *ν*max ($CHCl_3$) 3470, 1740, 1715, 1640; ^{1}H NMR (*δ*, $CDCl_3$) 6.21 (1H, d, *J* = 7.9 Hz, H-14), 5.89 (1H, dd, J_1 = 6.6 Hz, J_2 = 7.9 Hz, H-15), 3.88 (1H, d, *J* = 11.4 Hz, H-17), 3.80 (1H, d, *J* = 11.4 Hz, H-17), 3.21 (1H, d, J_1 = 4.6 Hz, J_2 = 11.5 Hz, H-3), 3.14 (1H, d, *J* = 6.6 Hz, H-12), 2.63 (1H, ddd, $J_1 = J_2$ = 3.3 Hz, J_3 = 13.7 Hz, H-1), 2.07 (3H, s, MeCOO), 1.94 (1H, ddd, $J_1 = J_2$ = 5.0 Hz, J_3 = 11.4 Hz, H-7), 1.77 (1H, s, H-9), 0.97, 0.74, 0.67 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 37.7 (C-1), 27.2 (C-2), 78.6 (C-3), 39.1 (C-4), 54.5 (C-5), 18.6 (C-6), 36.6 (C-7), 39.3 (C-8), 59.0 (C-9), 41.2 (C-10), 210.0 (C-11), 60.7 (C-12), 122.9 (C-13), 141.9 (C-14), 47.3 (C-15), 75.5 (C-16), 68.8 (C-17), 28.5 (C-18), 17.3 (C-19), 15.6 (C-20), 20.9 (*Me*CO), 170.9 (Me*CO*); CIMS (CH_4) *m/z* 377 $[M + 1]^+$ (6), 317 (80), 299 (35), 281 (100); HRMS (CI) calcd for $C_{22}H_{32}O_5$ 377.2328, found 377.2315.

***ent*-3*β*,16α,17-Trihydroxyatis-13-en-11-one (18):** mp 217−219 °C; $[\alpha]^{25}_D$ −1° (*c* 0.5, CH_3OH); IR *ν*max ($CHCl_3$) 3490, 1720, 1645, 740; ^{1}H NMR (*δ*, MeOD) 6.24 (1H, d, *J* = 7.9 Hz, H-14), 5.92 (1H, dd, J_1 = 6.8 Hz, J_2 = 7.9 Hz, H-15), 3.29 (1H, d, *J* = 11.4 Hz, H-17), 3.22 (1H, d, *J* = 11.4 Hz, H-17), 3.19 (1H, d, J_1 = 4.7 Hz, J_2 = 11.5 Hz, H-3), 3.17 (1H, d, *J* = 6.8 Hz, H-12), 2.66 (1H, ddd, $J_1 = J_2$ = 3.5 Hz, J_3 = 13.7 Hz, H-1), 1.95 (1H, ddd, $J_1 = J_2$ = 2.4 Hz, J_3 = 12.7 Hz, H-7), 1.77 (1H, s, H-9), 1.00, 0.76, 0.72 (3H each, s); ^{13}C NMR (*δ*, MeOD) 39.2 (C-1), 27.8 (C-2), 79.3 (C-3), 40.2 (C-4), 55.9 (C-5), 19.8 (C-6), 37.9 (C-7), 40.4 (C-8), 62.3 (C-9), 42.6 (C-10), 212.8 (C-11), 60.0 (C-12), 124.3 (C-13), 142.8 (C-14), 48.5 (C-15), 77.8 (C-16), 68.7 (C-17), 29.0 (C-18),

17.7 (C-19), 16.3 (C-20); CIMS (CH_4) *m/z* 335 $[M + 1]^+$ (4), 317 (40), 299 (15), 281 (100); HRMS (CI) calcd for $C_{20}H_{30}O_4$ 335.2222, found 335.2230.

Biotransformation of *ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (8) with *Rhizopus nigricans*. Substrate **8** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 12 days, and the metabolites were recovered and chromatographed on Si to obtain **19** (250 mg, 80%), **20** (16 mg, 5%), and starting material (15 mg, 5%).

***ent*-7α-Acetoxy-3*β*,12α,17-trihydroxybeyer-15-ene (19):** mp 196−198 °C; $[\alpha]^{25}_D$ 51° (*c* 1, CH_3OH); IR *ν*max ($CHCl_3$) 3502, 1712, 1247; ^{1}H NMR (*δ*, MeOD) 5.82 (1H, d, *J* = 5.9 Hz, H-16), 5.77 (1H, d, *J* = 5.9 Hz, H-15), 4.90 (1H, dd, $J_1 = J_2 = 2.7$ Hz, H-7), 3.89 (1H, br s, H-12), 3.64 (1H, d, *J* = 10.9 Hz, H-17), 3.45 (1H, d, *J* = 10.9 Hz, H-17), 3.18 (1H, dd, $J_1 = J_2 = 7.0$ Hz, H-3), 2.05 (3H, s, MeCOO), 0.91, 0.78, 0.75 (3H each, s); ^{13}C NMR (*δ*, MeOD) 38.3 (C-1), 27.8 (C-2), 79.5 (C-3), 37.8 (C-4), 48.7 (C-5), 26.0 (C-6), 77.4 (C-7), 55.4 (C-8), 45.7 (C-9), 39.4 (C-10), 30.2 (C-11), 68.1 (C-12), 53.9 (C-13), 45.2 (C-14), 136.4 (C-15), 136.1 (C-16), 66.4 (C-17), 28.7 (C-18), 16.3 (C-19), 14.4 (C-20), 21.2 (*Me*CO), 172.3 (Me*CO*); CIMS (CH_4) *m/z* 379 $[M + 1]^+$ (2), 361 (57), 301 (100), 283 (60); HRMS (CI) calcd for $C_{22}H_{34}O_5$ 379.2484, found 379.2476.

***ent*-7α-Acetoxy-3*β*,12α,17-trihydroxy-15α,16α-epoxybeyerane (20):** mp 140−142 °C; $[\alpha]^{25}_D$ 19° (*c* 0.5, CH_3OH); IR *ν*max ($CHCl_3$) 3469, 1735, 1256; ^{1}H NMR (*δ*, MeOD) 3.32 (1H, d, *J* = 2.9 Hz, H-16), 3.46 (1H, d, *J* = 2.9 Hz, H-15), 5.07 (1H, dd, $J_1 = J_2 = 2.8$ Hz, H-7), 4.11 (1H, br s, H-12), 3.69 (1H, d, *J* = 10.9 Hz, H-17), 3.44 (1H, d, *J* = 10.9 Hz, H-17), 3.18 (1H, dd, $J_1 = 6.2$ Hz, $J_2 = 8.4$ Hz, H-3), 2.02 (3H, s, MeCOO), 0.97, 0.91, 0.80 (3H each, s); ^{13}C NMR (*δ*, MeOD) 38.6 (C-1), 27.8 (C-2), 79.4 (C-3), 38.0 (C-4), 48.7 (C-5), 25.9 (C-6), 75.0 (C-7), 51.0 (C-8), 49.2 (C-9), 39.4 (C-10), 29.3 (C-11), 67.9 (C-12), 49.3 (C-13), 31.7 (C-14), 57.1 (C-15), 55.4 (C-16), 63.2 (C-17), 28.6 (C-18), 16.1 (C-19), 15.8 (C-20), 21.1 (*Me*CO), 172.1 (Me*CO*); CIMS (CH_4) *m/z* 395 $[M + 1]^+$ (6), 377 (29), 335 (56), 317 (100); HRMS (CI) calcd for $C_{22}H_{34}O_6$ 395.2433, found 395.2422.

Biotransformation of *ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (8) with *Fusarium moniliforme*. Substrate **8** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 17 days, and the metabolites were recovered and chromatographed on Si to obtain **21** (10 mg, 3%), **11** (16 mg, 5%), and starting material (255 mg, 85%).

***ent*-7α-Acetoxy-12α,17-dihydroxy-15α,16α-epoxybeyerane (21):** mp 192−194 °C; $[\alpha]^{25}_D$ 20° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3436, 1734, 1250; ^{1}H NMR (*δ*, $CDCl_3$) 3.14 (1H, d, *J* = 3.1 Hz, H-16), 3.37 (1H, d, *J* = 3.1 Hz, H-15), 5.11 (1H, dd, $J_1 = J_2 = 2.7$ Hz, H-7), 4.17 (1H, dd, $J_1 = J_2 = 2.3$ Hz, H-12), 3.80 (1H, d, *J* = 10.8 Hz, H-17), 3.75 (1H, d, *J* = 10.8 Hz, H-17), 2.02 (3H, s, MeCOO), 0.89, 0.80, 0.78 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 38.9 (C-1), 18.4 (C-2), 41.8 (C-3), 32.6 (C-4), 47.9 (C-5), 25.1 (C-6), 73.4 (C-7), 49.3 (C-8), 47.7 (C-9), 37.2 (C-10), 28.3 (C-11), 70.0 (C-12), 48.1 (C-13), 30.9 (C-14), 56.0 (C-15), 54.2 (C-16), 65.7 (C-17), 33.2 (C-18), 21.6 (C-19), 15.5 (C-20), 21.3 (*Me*CO), 170.5 (Me*CO*); CIMS (CH_4) *m/z* 379 $[M + 1]^+$ (3), 361 (8), 319 (100), 301 (25); HRMS (CI) calcd for $C_{22}H_{34}O_5$ 379.2484, found 379.2469.

Epoxidation of *ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (8). Product **8** (50 mg) was epoxidized with 50 mg of *m*-CPBA in 3 mL of $CHCl_3$ for 24 h at room temperature. The reaction mixture was then diluted with $CHCl_3$; washed with aqueous $FeSO_4$, aqueous $NaHCO_3$, and H_2O; dried; and concentrated. Purification by flash chromatography provided **21** (47 mg, 90%), identical to that previously obtained by the biotransformation of **8** with *F. moniliforme*.

Biotransformation of *ent*-7α-Acetoxy-17-hydroxybeyer-15-en-12-one (11) with *Rhizopus nigricans*. Substrate **11** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 12 days, and the metabolites were recovered and chromatographed on Si to obtain **22** (245 mg, 78%), **23** (21 mg, 6.5%), and starting material (21 mg, 7%).

***ent*-7α-Acetoxy-3*β*,17-dihydroxybeyer-15-en-12-one (22):** mp 201−203 °C; $[\alpha]^{25}_D$ −282° (*c* 1, $CHCl_3$); IR *ν*max ($CHCl_3$) 3484, 1720, 1257; ^{1}H NMR (*δ*, $CDCl_3$) 5.98 (1H, d, *J* = 5.7 Hz, H-16), 5.91 (1H, d, *J* = 5.7 Hz, H-15), 5.05 (1H, dd, $J_1 = J_2 = 2.7$ Hz, H-7), 3.65 (1H, d, *J* = 11.7 Hz, H-17), 3.54 (1H, d, *J* = 11.7 Hz, H-17), 3.18 (1H, dd, $J_1 = 4.7$ Hz, $J_2 = 11.4$ Hz, H-3), 2.00 (3H, s, MeCOO), 0.87, 0.72, 0.72 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 36.2 (C-1), 26.7 (C-2), 78.2 (C-3), 36.9 (C-4), 46.8 (C-5), 24.8 (C-6), 74.1 (C-7), 52.2 (C-8), 49.4 (C-9), 38.1 (C-10), 36.1 (C-11), 212.6 (C-12), 62.8 (C-13), 49.3 (C-14), 138.1 (C-15), 133.4 (C-16), 63.4 (C-17), 27.8 (C-18), 15.3 (C-19), 13.4 (C-20), 21.1 (*Me*CO), 170.2 (Me*CO*); CIMS (CH_4) *m/z* 377 $[M + 1]^+$ (3), 359 (22), 341 (35), 281 (100); HRMS (CI) calcd for $C_{22}H_{32}O_5$ 377.2328, found 377.2323.

***ent*-7α-Acetoxy-3*β*,17-dihydroxy-15α,16α-epoxybeyeran-12-one (23):** mp 254−256 °C; $[\alpha]^{25}_D$ −145° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3448, 1737, 1712, 1242; ^{1}H NMR (*δ*, $CDCl_3$) 3.59 (1H, d, *J* = 3.0 Hz, H-16), 3.62 (1H, d, *J* = 3.0 Hz, H-15), 5.28 (1H, dd, $J_1 = J_2 = 2.7$ Hz, H-7), 3.83 (1H, d, *J* = 11.8 Hz, H-17), 3.66 (1H, d, *J* = 11.8 Hz, H-17), 3.25 (1H, dd, $J_1 = 4.7$ Hz, $J_2 = 11.3$ Hz, H-3), 2.02 (3H, s, MeCOO), 0.99, 0.93, 0.80 (3H each, s); ^{13}C NMR (*δ*, $CDCl_3$) 36.9 (C-1), 26.9 (C-2), 78.3 (C-3), 38.3 (C-4), 46.9 (C-5), 24.7 (C-6), 72.0 (C-7), 47.7 (C-8), 52.4 (C-9), 36.9 (C-10), 37.4 (C-11), 211.4 (C-12), 59.2 (C-13), 35.3 (C-14), 55.2 (C-15), 54.1 (C-16), 61.4 (C-17), 27.9 (C-18), 15.6 (C-19), 15.4 (C-20), 21.1 (*Me*CO), 169.9 (Me*CO*); CIMS (CH_4) *m/z* 393 $[M + 1]^+$ (3), 375 (10), 357 (42), 297 (100); HRMS (CI) calcd for $C_{22}H_{32}O_6$ 393.2277, found 393.2272.

Biotransformation of *ent*-7α-Acetoxy-17-hydroxybeyer-15-en-12-one (11) with *Fusarium moniliforme*. Substrate **11** (300 mg) was dissolved in EtOH (5 mL) and the solution distributed among five Erlenmeyer flask cultures. These were incubated for 17 days, and the metabolites were recovered and chromatographed on Si to obtain **24** (9 mg, 3%), **25** (12.5 mg, 4%), **26** (14 mg, 5%), and starting material (240 mg, 80%).

***ent*-7α-Acetoxy-17-hydroxy-15α,16α-epoxybeyeran-12-one (24):** mp 226−228 °C; $[\alpha]^{25}_D$ −72° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3590, 1738, 1711, 1237; ^{1}H NMR (*δ*, $CDCl_3$) 3.58 (1H, d, *J* = 3.0 Hz, H-16), 3.64 (1H, d, *J* = 3.0 Hz, H-15), 5.27 (1H, dd, $J_1 = J_2 = 2.8$

Hz, H-7), 3.83 (1H, dd, $J_1 = 5.3$ Hz, $J_2 = 11.8$ Hz, H-17), 3.66 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 11.8$ Hz, H-17), 2.41 (1H, dd, $J_1 = 7.0$ Hz, $J_2 = 16.3$ Hz, H-11), 2.34 (1H, dd, $J_1 = 11.7$ Hz, $J_2 = 16.3$ Hz, H-11), 2.02 (3H, s, MeCOO), 0.99, 0.84, 0.82 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 37.5 (C-1), 18.3 (C-2), 41.6 (C-3), 32.6 (C-4), 47.8 (C-5), 24.9 (C-6), 72.3 (C-7), 47.9 (C-8), 52.6 (C-9), 37.8 (C-10), 38.6 (C-11), 211.8 (C-12), 59.2 (C-13), 35.5 (C-14), 55.2 (C-15), 54.3 (C-16), 61.5 (C-17), 33.1 (C-18), 21.5 (C-19), 15.6 (C-20), 21.2 (*Me*CO), 170.0 (Me*CO*); CIMS (CH_4) *m/z* 377 $[M + 1]^+$ (5), 359 (28), 341 (17), 297 (100); HRMS (CI) calcd for $C_{22}H_{32}O_5$ 377.2328, found 377.2327.

***ent*-7α-Acetoxy-1*β*,17-dihydroxybeyer-15-en-12-one (25):** mp 126−128 °C; $[\alpha]^{25}_D$ −319° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3455, 1735, 1707, 1241; ^{1}H NMR (δ, $CDCl_3$) 6.01 (1H, d, $J = 5.8$ Hz, H-16), 5.95 (1H, d, $J = 5.8$ Hz, H-15), 5.05 (1H, dd, $J_1 = J_2 = 2.8$ Hz, H-7), 3.68 (1H, dd, $J_1 = 5.7$ Hz, $J_2 = 11.4$ Hz, H-17), 3.60 (1H, dd, $J_1 = 7.4$ Hz, $J_2 = 11.4$ Hz, H-17), 3.35 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 10.5$ Hz, H-1), 2.79 (1H, dd, $J_1 = 7.0$ Hz, $J_2 = 17.8$ Hz, H-11), 2.66 (1H, dd, $J_1 = 9.9$ Hz, $J_2 = 17.8$ Hz, H-11), 2.05 (3H, s, MeCOO), 0.82, 0.81, 0.80 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 79.3 (C-1), 29.5 (C-2), 39.7 (C-3), 32.5 (C-4), 50.1 (C-5), 24.9 (C-6), 74.5 (C-7), 53.0 (C-8), 46.4 (C-9), 42.9 (C-10), 39.9 (C-11), 213.7 (C-12), 62.7 (C-13), 49.3 (C-14), 138.1 (C-15), 133.9 (C-16), 64.0 (C-17), 32.9 (C-18), 21.2 (C-19), 9.3 (C-20), 21.1 (*Me*CO), 170.3 (Me*CO*); CIMS (CH_4) *m/z* 377 $[M + 1]^+$ (4), 359 (23), 341 (24), 297 (100); HRMS (CI) calcd for $C_{22}H_{32}O_5$ 377.2328, found 377.2335.

***ent*-1*β*,7α,17-Trihydroxybeyer-15-en-12-one (26):** mp 92−94 °C; $[\alpha]^{25}_D$ −146° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3321, 1702, 1242; ^{1}H NMR (δ, $CDCl_3$) 5.98 (1H, d, $J = 5.7$ Hz, H-16), 5.94 (1H, d, $J = 5.7$ Hz, H-15), 3.90 (1H, dd, $J_1 = J_2 = 2.9$ Hz, H-7), 3.76 (1H, d, $J = 11.6$ Hz, H-17), 3.57 (1H, d, $J = 11.6$ Hz, H-17), 3.35 (1H, dd, $J_1 = 5.1$ Hz, $J_2 = 10.5$ Hz, H-1), 2.77 (1H, dd, $J_1 = 7.1$ Hz, $J_2 = 17.8$ Hz, H-11), 2.65 (1H, dd, $J_1 = 9.7$ Hz, $J_2 = 17.8$ Hz, H-11), 0.88, 0.83, 0.81 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 79.3 (C-1), 29.6 (C-2), 39.8 (C-3), 32.5 (C-4), 48.7 (C-5), 27.9 (C-6), 72.0 (C-7), 54.4 (C-8), 45.1 (C-9), 43.0 (C-10), 39.9 (C-11), 214.1 (C-12), 62.7 (C-13), 49.6 (C-14), 139.1 (C-15), 133.4 (C-16), 64.1 (C-17), 32.8 (C-18), 21.4 (C-19), 9.3 (C-20); CIMS (CH_4) *m/z* 335 $[M + 1]^+$ (6), 317 (44), 299 (100), 281 (42); HRMS (CI) calcd for $C_{20}H_{30}O_4$ 335.2222, found 335.2238.

Epoxidation of *ent*-7α-Acetoxy-12α,17-dihydroxybeyer-15-ene (11). Product **11** (50 mg) was epoxidized with 50 mg of *m*-CPBA in 3 mL of $CHCl_3$ for 2 h at room temperature. The reaction mixture was then diluted with $CHCl_3$; washed with aqueous $FeSO_4$, aqueous $NaHCO_3$, and H_2O; dried; and concentrated. Purification by flash chromatography provided **24** (18 mg, 35%), identical to that previously obtained by the biotransformation of **11** with *F. moniliforme*, and **27** (23 mg, 45%).

***ent*-7α-Acetoxy-17-hydroxy-15α,16α-epoxybeyeran-13,12-olide (27):** mp 126−128 °C; $[\alpha]^{25}_D$ 26° (*c* 0.5, $CHCl_3$); IR *ν*max ($CHCl_3$) 3428, 1734, 1710, 1258; ^{1}H NMR (δ, $CDCl_3$) 5.78 (1H, d, $J = 5.2$ Hz, H-16), 3.78 (1H, d, $J = 5.2$ Hz, H-15), 5.15 (1H, dd, $J_1 = J_2 = 2.7$ Hz, H-7), 3.77 (2H, br s, H-17), 2.82 (1H, dd, $J_1 = 2.9$ Hz, $J_2 = 13.4$ Hz, H-11), 2.69 (1H, dd, $J_1 = J_2 = 13.4$ Hz, H-11), 2.09 (3H, s, MeCOO), 1.00, 0.88, 0.87 (3H each, s); ^{13}C NMR (δ, $CDCl_3$) 39.3 (C-1), 18.5 (C-2), 41.3 (C-3), 32.8 (C-4), 46.9 (C-5), 24.8 (C-6), 74.1 (C-7), 48.9 (C-8), 51.0 (C-9), 38.4 (C-10), 34.0 (C-11), 174.1 (C-12), 86.1 (C-13), 42.9 (C-14), 58.8 (C-15), 56.1 (C-16), 65.6 (C-17), 33.1 (C-18), 21.5 (C-19), 15.4 (C-20), 21.2 (*Me*CO), 169.7 (Me*CO*); CIMS (CH_4) *m/z* 393 $[M + 1]^+$ (4), 375 (56), 315 (100); HRMS (CI) calcd for $C_{22}H_{32}O_6$ 393.4925, found 393.4933.

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