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Biotransformation of Shiromool Derivatives by *Rhizopus nigricans* Cultures: Chemical-Microbiological Synthesis of Michelenolide Analogues

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Abstract: The biotransformation of two derivatives of shiromool was carried out with *Rhizopus nigricans* cultures to obtain 11- and 12- hydroxylated metabolites. The 11-hydroxyl germacrane compound was transformed to 11-R and 11-S 12-hydroxyl derivatives which were oxidised with TPAP to give michelenolide analogues. In the biotransformation processes, 2β- 3α- and 9β-hydroxyl derivatives were also obtained, as well as a 2β,11-dihydroxylated metabolite.

INTRODUCTION

As part of a series of studies on ways to obtain sesquiterpene lactones from a non-lactone sesquiterpene compound, we carried out the semisynthesis of germacrane lactones from derivatives of shiromool[1], an anti-insect feeding compound.

Sesquiterpene lactones are a wide and varied group of compounds with biological activity[2-7]. 6β-sesquiterpenolides, which are scarce in nature and have rarely been studied, are of interest because of their role as possible intermediates in some biogenetic pathways[3]. In biogenetic terms sesquiterpene lactones are formed from an initial hydroxylation at C-12 which evolves to a carboxylic acid. Subsequent enzymatically-mediated hydroxylation at C-6 or C-8 (and lactonization) yield the respective sesquiterpenolides[8-10]. In previous papers, we reported the synthesis of 6β-eudesmanolides by chemical-microbiological means[11,12], with the help of the microorganisms *Curvularia lunata* and *Rhizopus nigricans*. Also, we have developed a method to epimerize 6α-sesquiterpene lactones[13], more abundant in nature, to the corresponding 6β-sesquiterpenolides. The use of a microbiological pathway to obtain 11- and 12-hydroxyl derivatives of shiromool acetate (**1**) allowed us to obtain some michelenolide[14] analogues.

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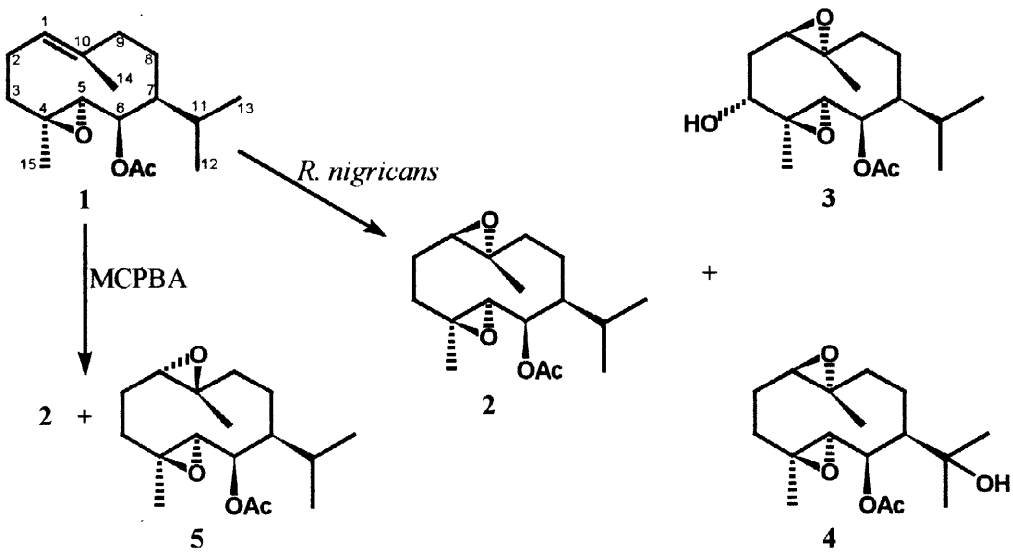
RESULTS AND DISCUSSION

Compound **1** (shiromool acetate), isolated from *Sideritis varoi* subspecies *cuatrecasasii*[15], was incubated with a culture of *Rhizopus nigricans* during 6 days. After the recovery and purification of the metabolites, a major product was isolated (**2**, 58%) together with two more polar minor products (**3**, 6%) and (**4**, 10%).

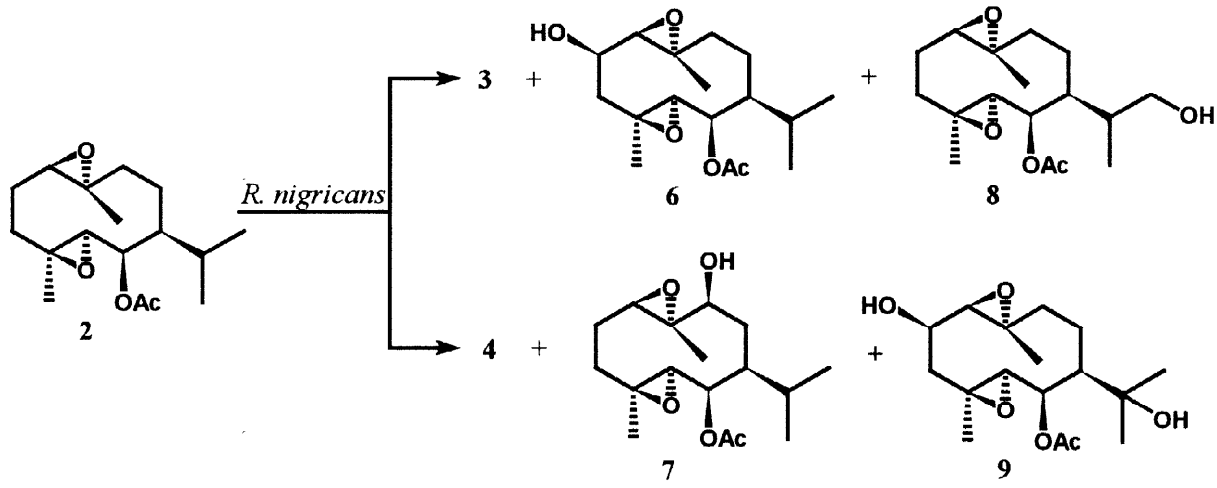


The first metabolite isolated (**2**) (molecular formula $C_{17}H_{28}O_4$) lacked double bond signals in its ^{1}H NMR spectrum; a signal appeared at δ 2.99 (1H, dd, J_1= 9.2, J_2= 5.8 Hz, H-1) that corresponded to a geminal proton to an oxygenated function. In the ^{13}C NMR spectrum two new signals appeared representing oxygenated carbons, one of them methyne (δ 61.49) and the other one completely substituted (δ 61.85). These data were compatible with the epoxidation of the double bond of substrate (**1**) by the microorganism. To determine the configuration of the carbons C-1 and C-10 we carried out epoxidation of compound **1** with m-chloroperbenzoic acid (MCPBA), from which a major epoxide identical to metabolite **2** was obtained together with a minor epoxide (**5**). By comparing the NMR spectra of both products with those described for the epoxides of shiromool[16], we assigned a (1β,10α)-epoxy arrangement for the diepoxide **2** and a (1α,10β)-epoxy arrangement for the diepoxide **5**.

The ^{1}H NMR spectrum of the second metabolite (**3**) was similar to that of metabolite **2**, with a new signal appearing at δ 2.95 (1H, dd, J_1= 8.7, J_2= 6.4 Hz) due to a geminal proton to a hydroxyl group that was probably situated on C-3 or C-9. Analysis of the ^{13}C NMR spectra of both metabolites (**2** and **3**) helped to establish the position of this new hydroxylation on C-3. The absolute configuration of this carbon was determined by the Horeau method[17] as "R", which implied an α-hydroxylation. Accordingly we assigned the structure of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-3α-hydroxygermacrane to metabolite **3**.

On comparing the ^{1}H NMR spectra of metabolites **2** and **4**, we observed that the doublet signals of the methyl groups at C-11 in metabolite **2** had become singlet signals in metabolite **4**. Therefore a new hydroxyl group seemed to be located at C-11. This was confirmed by analysis of the ^{13}C NMR spectral data of the two metabolites. In conclusion, metabolite **4** had a 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-11-hydroxygermacrane structure.

The action of the microorganism was mainly directed to the selective epoxidation of the double bond of the substrate, and then to the hydroxylation of certain positions of the molecule. To obtain higher yields of hydroxylated metabolites, we incubated diepoxide **2**, obtained from **1** by *Rhizopus nigricans*. After incubation for 12 days we isolated 17% of unaltered substrate (**2**), metabolites **3** (18%) and **4** (25%), and four new metabolites: **6** (10%), **7** (9%), **8** (6%) and **9** (2%).

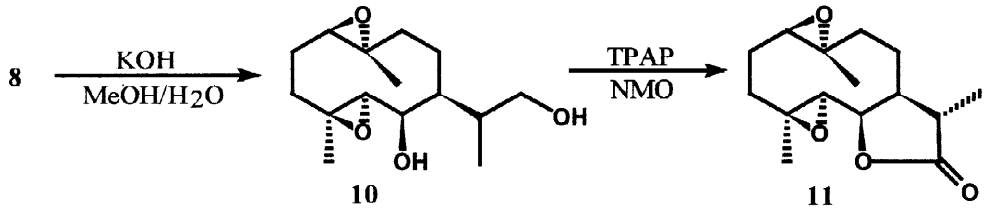


The molecular formula of metabolites **6**, **7** and **8** was $C_{17}H_{28}O_5$. In the ^{1}H NMR spectrum of metabolite **6** a new signal appeared at δ 3.82 (ddd, J_1= 7.3, J_2= 6.5, J_3= 1.2 Hz) and the H-1 signal appeared as a doublet (δ 3.08, J= 7.3 Hz). Double resonance experiments on these signals allowed us to locate the new hydroxylation on carbon 2. The stereochemistry of this hydroxylation was determined by the Horeau method as "R". Therefore we assigned the structure of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-2β-hydroxygermacrane to metabolite **6**.

In the ^{1}H NMR spectrum of metabolite **7** a signal appeared at δ 3.08 due to a geminal proton to a hydroxyl group coupled to two other protons (J_1= 11.7, J_2= 2.0 Hz). This hydroxylation was located on C-9, as confirmed by its ^{13}C NMR data. The configuration of this carbon was established by the Horeau method as "S", so the arrangement of the hydroxyl group was β, and metabolite **7** was thus identified as 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-9β-hydroxygermacrane.

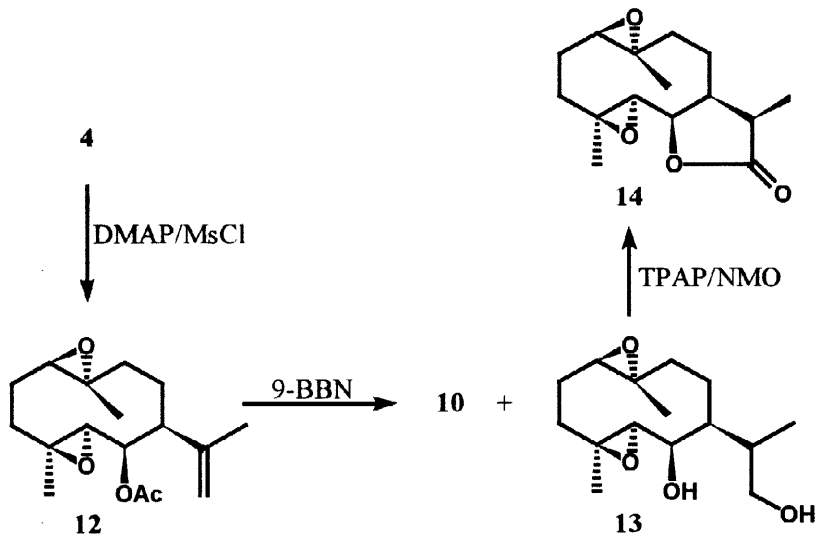
The ^{1}H NMR spectrum of metabolite **8** differed from that of metabolite **2** in the disappearance of the signal of one methyl from the isopropyl group, and the appearance of two new signals centred at δ 3.58 and 3.48 that constituted the AB part of an ABX system. These data were compatible with the hydroxylation of one

methyl of the isopropyl group, as confirmed by ^{13}C NMR. Saponification of metabolite **8** led to product **10**, whose spectroscopic data were compatible with a structure of (1β,10α),(4β,5α)-diepoxy-6β,12-dihydroxygermacrane. Treatment of this dihydroxylated derivative (**10**) with tetrapropylammonium perruthenate[18] (TPAP) gave rise to a product with a structure of (1β,10α),(4β,5α)-diepoxygermacran-6β,12-olide (**11**), which was the result of oxidation of the hydroxyl group at C-12 and lactonization. The C-11 configuration was determined from the value of the coupling constants of the H-11 signal (δ 2.45, c, $J_{11,13}$= 7.6 Hz), which indicated that H-7 and H-11 were not coupled because the dihedral angle formed by these protons was close to 90°. These results were compatible with an 11S configuration for lactone **11**, whose structure was (1β,10α),(4β,5α)-diepoxy-7α,11β-H-germacran-6β,12-olide; therefore, their precursor (**8**) was (11S)-6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-12-hydroxygermacrane.



The molecular formula of the more polar product isolated from this incubation (**9**), was $C_{17}H_{28}O_6$. Its ^1H NMR spectrum showed signals similar to those of metabolites **4** and **6**, so we deduced that metabolite **9** was 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-2β,11-dihydroxygermacrane.

Starting from the 11-hydroxyl derivative (**4**), the major product isolated from the incubation of the diepoxygermacrane **2** with *Rhizopus nigricans*, we accomplished the semisynthesis of a new 6β-germacranolide. The hydroxyl group at C-11 was dehydrated through the formation of the corresponding mesylate using 4-dimethylaminopyridine (DMAP) and methanesulfonyl chloride[19]; these gave rise to the elimination product with the less-substituted double bond (**12**), i. e. 6β-acetoxy-(1β,10α),(4β,5α)-diepoxygermacr-11-ene. The 12-hydroxyl derivatives were formed by hydroboration of product **12** with 9-borabicycle[3.3.1]nonane (9-BBN), to give rise to a diol mixture (84%) from which the previously obtained product **10** was isolated (24%) together with the epimer at C-11 (**13**, 60%). Both products were produced from hydration on both faces of the double bond and saponification of the acetoxyl group at C-6. Treatment of product **13** with TPAP led to product **14**. Its ^1H NMR spectrum was very similar to that of lactone **11**, differing only in the chemical shift and the form of the H-11 proton signal. This signal showed greater deshielding (Δδ= 0.4) and appeared as a double quadruplet. Protons H-7 and H-11 were coupled at a dihedral angle of approximately 40°. Therefore we assigned the configuration 11R to this product (**14**), with a structure of (1β,10α),(4β,5α)-diepoxy-7α,11α-H-germacran-6β,12-olide. Thus product **13** was (11R)-(1β,10α),(4β,5α)-diepoxy-6β,12-dihydroxygermacrane.



The isolation of metabolites with hydroxylations at C-11 or C-12 is of interest as they are appropriate precursors for conversion to 6β-sesquiterpenolides.

EXPERIMENTAL

Measurements of NMR spectra (300.13 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal) in a BRUKER AM-300 or ARX-400 spectrometers. The assignments of ^{13}C chemical shifts were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Bruker's programs were used for COSY (45°) and C/H correlation. Monodimensional n.O.e.-difference experiments were made by irradiation for 4 seconds in series of 8 scans. Ir spectra were recorded on a Nicolet 20SX FT-IR spectrometer. Mass spectra were determined with CI (methane) in a Hewlett-Packard 5988A spectrometer. High resolution mass spectra were made in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Mps were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°. Silica gel Scharlau 60 (40-60 μm) was used for flash chromatography. CH_2Cl_2 or $CHCl_3$ containing increasing amounts of Me_2CO were used as eluents. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with H_2SO_4-AcOH, followed by heating to 120°. The identity of compound **1** was confirmed by direct comparison with an authentic sample (IR, MS, NMR, etc.).

Isolation of 6β-acetoxy-4β,5α-epoxy-trans-germacr-1(10)-ene (1). 6β-acetoxy-4β,5α-epoxy-*trans*-germacr-1(10)-ene was isolated from *Sideritis varoi* subspecies *cuatrecasasii*[15].

Organism, media and culture conditions. *Rhizopus nigricans* CECT 2072 was obtained from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain, and was kept in YEPGA medium containing yeast extract (1%), peptone (1%), glucose (2%) and agar (2%) in H_2O at pH 5. In all transformation experiments a medium of peptone (0.1%), yeast extract (0.1%), beef extract (0.1%) and glucose (0.5%) in H_2O at pH 5.7 was used. Erlenmeyer flasks (250 ml) containing 80 ml of medium were inoculated with a dense suspension of *R. nigricans*. The cultures were incubated by shaking (150 rpm) at 28° for 6 days, after which substrates **1** and **2** in EtOH were added.

Biotransformation of substrate 1. Substrate **1** (250 mg) was dissolved in EtOH (5ml), distributed among 5 Erlenmeyer-flask cultures and incubated for 6 days, after which the cultures were filtered and pooled; the cells were washed thoroughly with water and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Both extracts were pooled, dried with anhydrous Na_2SO_4, and evaporated at 40° in vacuum to give a mixture of compounds (223 mg). This mixture was chromatographed on a silica gel column to obtain 22 mg of starting material **1**, 154mg (58%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxygermacrane (**2**); syrup; $[\alpha]_D$= -66° ($CHCl_3$, c 1); IR (film): 1734 and 1244 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 and 0.89 (3H each, d, J= 6.4 Hz, 3H-12 and 3H-13), 1.30 (3H, s, 3H-14), 1.41 (3H, s, 3H-15), 2.04 (3H, s, AcO group), 2.89 (1H, d, J= 8.5 Hz, H-5), 2.99 (1H, dd, $J_{1\alpha,2\beta}$= 9.2, $J_{1\alpha,2\alpha}$= 5.8 Hz, H-1) and 5.0 (1H, dd, $J_{6,5}$= 8.5, $J_{6,7}$= 1.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 16.75 (C-14), 16.78 (C-15), 20.56 (C-13), 20.62 (C-12), 21.10 (*Me*CO), 23.49 (C-8), 24.70 (C-2), 31.81 (C-11), 34.30 (C-3), 40.78 (C-9), 48.76 (C-7), 59.24 (C-4), 59.86 (C-10), 60.07 (C-1), 65.76 (C-5), 72.73 (C-6) and 170.12 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 297.2061, ($C_{17}H_{28}O_4$ 297.2066, PPM 1.6); 18 mg (6%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-3α-hydroxygermacrane (**3**); syrup; $[\alpha]_D$= -43° ($CHCl_3$, c 1); IR (film): 3450, 1741, 1242 and 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 and 0.91 (3H each, d, J= 6.6 Hz, 3H-12 and 3H-13), 1.32 and 1.44 (3H each, s, 3H-14 and 3H-15), 2.08 (3H, s, AcO group), 2.94 (1H, d, J= 8.4 Hz; H-5), 2.95 (1H, dd, $J_{3\beta,2\alpha}$= 8.7, $J_{3\beta,2\beta}$= 6.4 Hz, H-3β), 3.32 (1H, dd, $J_{1,2\beta}$= 10.1, $J_{1,2\alpha}$= 8.2 Hz, H-1) and 5.06 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 11.30 (C-15), 17.15 (C-14), 20.60 (C-13), 20.65 (C-12), 21.13 (*Me*CO), 23.49 (C-8), 31.88 (C-11), 33.27 (C-2), 40.68 (C-9), 48.62 (C-7), 58.21 (C-1), 59.69 (C-10), 62.07 (C-4), 63.98 (C-5), 72.26 (C-6), 74.94 (C-3) and 170.16 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 313.2013, ($C_{17}H_{28}O_5$ 313.2015, PPM 0.7); Determination of absolute configuration at C-3: 10 mg of metabolite **3** and 25 mg of racemic α-phenylbutyric anhydride (dissolved in 1 ml of pyridine)[17], $[\alpha]_D$= +14° ($CHCl_3$, c 5); and 29 mg (10%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-11-hydroxygermacrane (**4**); syrup; $[\alpha]_D$= -38° ($CHCl_3$, c 1); IR (film): 3482, 1738, 1237 and 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.14 and 1.18 (3H each, s, 3H-12 and 3H-13), 1.33 and 1.43 (3H each, s, 3H-14 and 3H-15), 2.07 (3H, s, AcO group), 2.92 (1H, d, J= 8.4 Hz, H-5), 3.00 (1H, dd, $J_{1,2\beta}$= 9.1, $J_{1,2\alpha}$= 5.8 Hz, H-1) and 5.19 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 16.79 (C-14 and C-15), 20.44 (C-8), 21.46 (*Me*CO), 24.63 (C-2), 27.07 (C-13), 28.37 (C-12), 34.22 (C-3), 41.16 (C-9), 51.14 (C-7), 59.57 (C-4), 59.84 (C-10), 60.29 (C-1), 65.91 (C-5), 72.29 (C-6), 73.09 (C-11) and 170.81 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 313.2018, ($C_{17}H_{28}O_5$ 313.2015, PPM -0.8).

Epoxidation of substrate 1. 3.0 g of 6β-acetoxy-4β,5α-epoxy-*trans*-germacr-1(10)-ene (**1**) were dissolved in 300 ml of $CHCl_3$ and 4 g of m-chloro-perbenzoic acid (MCPBA) were added. The mixture was stirred for 24

h at room temperature, then was diluted with 400 ml of $CHCl_3$ and washed successively with saturated solutions of $FeSO_4$ and $NaHCO_3$ and finally water. The organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. Chromatography over silica gel yielded 1.870 g (59%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxygermacrane (**2**) and 0.752 g (24%) of 6β-acetoxy-(1α,10β),(4β,5α)-diepoxygermacrane (**5**); syrup; $[\alpha]_D$= +81° ($CHCl_3$, c 1); IR (film): 1738 and 1241 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 and 0.98 (3H each, d, J= 6.6 Hz, 3H-12 and 3H-13) 1.30 and 1.44 (3H each, s, 3H-14 and 3H-15), 2.05 (3H, s, AcO group), 2.92 (1H, d, $J_{1,2\alpha}$= 10.6 Hz, H-1), 3.00 (1H, d, J= 7.1 Hz, H-5) and 4.95 (1H, dd, $J_{6,5}$= 7.1, $J_{6,7}$= 1.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 16.74 (C-14), 20.60 (C-13), 20.94 (C-12), 21.10 (*Me*CO), 23.17 (C-15), 23.75 (C-8), 25.97 (C-2), 31.05 (C-11), 36.54 (C-3), 36.62 (C-9), 44.22 (C-7), 58.81 (C-4), 61.49 (C-1), 61.85 (C-10), 66.23 (C-5), 73.54 (C-6) and 170.21 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 297.2072, ($C_{17}H_{29}O_4$ 297.2066, PPM -2.0).

Biotransformation of substrate 2. Substrate **2** (1.5 g) was dissolved in EtOH (30 ml), distributed among 30 Erlenmeyer flask cultures and incubated for 12 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. The resulting mixture (1.344 g) was chromatographed on a silica gel column to obtain 259 mg (17%) of starting material (**2**), 287 mg (18%) of metabolite **3**, 388 mg (25%) of metabolite **4**, 151 mg (10%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-2β-hydroxygermacrane (**6**); syrup; $[\alpha]_D$= -29° ($CHCl_3$, c 1); IR (film): 3452, 1740, 1234 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 and 0.92 (3H each, d, J= 6.5 Hz, 3H-12 and 3H-13), 1.32 (3H, s, 3H-15), 1.55 (3H, s, 3H-14), 2.07 (3H, s, AcO group), 2.85 (1H, d, J= 8.4 Hz, H-5), 3.08 (1H, d, J= 7.4 Hz, H-1), 3.82 (1H, bt, $w_{1/2}$= 14 Hz, H-2) and 5.05 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.4 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 17.40 (C-15), 18.58 (C-14), 20.62 (C-12 and C-13), 21.18 (*Me*CO), 23.43 (C-8), 31.90 (C-11), 41.12 (C-9), 43.94 (C-3), 48.87 (C-7), 58.44 (C-4), 60.02 (C-10), 65.59 (C-2), 65.67 (C-5), 70.08 (C-1), 72.80 (C-6) and 170.16 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 313.2020, ($C_{17}H_{28}O_5$ 313.2015, PPM -1.6); Determination of absolute configuration at C-2: 17 mg of metabolite **6** and 25 mg of racemic α-phenylbutyric anhydride (dissolved in 1 ml of pyridine)[17], $[\alpha]_D$= +12° ($CHCl_3$, c 2.5); 139 mg (9%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-9β-hydroxygermacrane (**7**), syrup; $[\alpha]_D$= -32° ($CHCl_3$, c 1); IR (film): 3454, 1741, 1242 and 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 and 0.96 (3H each, d, J= 6.6 Hz, 3H-12 and 3H-13), 1.36 and 1.42 (3H each, s, 3H-14 and 3H-15), 2.08 (3H, s, AcO group), 2.90 (1H, d, J= 8.4 Hz, H-5), 3.00 (1H, dd, $J_{1,2\beta}$= 9.1, $J_{1,2\alpha}$= 5.9 Hz, H-1), 3.08 (1H, dd, $J_{9\beta,8\alpha}$= 11.7, $J_{9\beta,8\beta}$= 2.0 Hz, H-9β) and 5.01 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.5 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 10.56 (C-14), 16.92 (C-15), 20.49 (C-13), 20.58 (C-12), 21.17 (*Me*CO), 24.21 (C-2), 32.04 (C-11), 32.27 (C-8), 34.07 (C-3), 45.58 (C-7), 58.68 (C-1), 58.98 (C-4), 62.27 (C-10), 65.52 (C-5), 72.74 (C-6), 80.74 (C-9) and 170.16 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 313.2021, ($C_{17}H_{28}O_5$ 313.2015, PPM -2.0); Determination of absolute configuration at C-9: 20 mg of metabolite **7** and 25 mg of racemic α-phenylbutyric anhydride (dissolved in 1 ml of pyridine)[17], $[\alpha]_D$= -15° ($CHCl_3$, c 2.5); 95 mg (6%) of (11S)-6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-12-hydroxygermacrane (**8**); syrup; $[\alpha]_D$= -33° ($CHCl_3$, c 1); IR (film): 3469, 1738, 1235 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.95 (3H, d, J= 6.3 Hz, 3H-13), 1.32 and 1.44 (3H each, s, 3H-14 and 3H-15), 2.07 (3H, s, AcO group), 2.91 (1H, d, J= 8.4 Hz, H-5), 3.05 (1H, dd, $J_{1,2\beta}$= 9.1, $J_{1,2\alpha}$= 5.8 Hz, H-1), 3.48 and 3.58 (1H each, part AB of an ABX system, J_{AB}= 10.6, J_{AX}= 4.2, J_{BX}= 3.5 Hz, 2H-12) and 5.02 (1H, d, J= 8.4 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 14.79 (C-13), 16.78 (C-14 and C-15), 21.25 (*Me*CO), 22.58 (C-8),

24.75 (C-2), 34.35 (C-3), 39.01 (C-11), 40.62 (C-9), 42.51 (C-7), 59.60 (C-4), 59.84 (C-10), 60.09 (C-1), 64.93 (C-12), 65.84 (C-5), 73.62 (C-6) and 170.47 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 313.2021, ($C_{17}H_{28}O_5$ 313.2015, PPM -1.9); and 25 mg (2%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-2β,11-dihydroxygermacrane (**9**); syrup; $[\alpha]_D$= -35° ($CHCl_3$, c 1); IR (film): 3472, 1739, 1235 and 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.17 and 1.20 (3H each, s, 3H-12 and 3H-13), 1.33 (3H, s, 3H-15), 1.56 (3H, s, 3H-14), 2.08 (3H, s, AcO group), 2.86 (1H, d, J= 8.4 Hz, H-5), 3.08 (1H, d, J= 7.3 Hz, H-1), 3.81 (1H, ddd, $J_{2,1}$= 7.3, $J_{2,3\beta}$= 6.5, $J_{2,3\alpha}$= 1.2 Hz, H-2) and 5.22 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 17.33 (C-15), 18.44 (C-14), 20.23 (C-8), 21.42 (*Me*CO), 27.27 (C-13), 27.42 (C-12), 41.27 (C-9), 43.86 (C-3), 51.08 (C-7), 58.78 (C-4), 60.28 (C-10), 65.92 (C-2), 66.02 (C-5), 69.41 (C-1), 72.24 (C-6), 73.11 (C-11) and 170.79 (Me*CO*); HRLSIMS, *m/z*: $[M+1]^+$ 329.1976, ($C_{17}H_{28}O_6$ 329.1964, PPM -3.6).

Saponification of product 8. (11S)-6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-12-hydroxygermacrane (**8**, 45 mg) was dissolved in $MeOH/H_2O$ (70%) (4 ml) containing KOH (5%) (0.2 g) and refluxed for 1 h. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded (11S)-(1β,10α),(4β,5α)-diepoxy-6β,12-dihydroxygermacrane (**10**, 34 mg, 87%); syrup; $[\alpha]_D$= -25° ($CHCl_3$, c 1); IR (film): 3462 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (3H, d, J= 7.0 Hz, 3H-13), 1.32 (3H, s, 3H-15), 1.39 (3H, s, 3H-14), 2.90 (1H, d, J= 8.5 Hz, H-5), 3.00 (1H, dd, $J_{1,2\beta}$= 9.2, $J_{1,2\alpha}$= 5.7 Hz, H-1), 3.70 (1H, dd, $J_{6,5}$= 8.5, $J_{6,7}$= 1.4 Hz, H-6) and 3.51 and 3.74 (1H each, part AB of an ABX system, J_{AB}= 11.1, J_{AX}= 2.7, J_{BX}= 5.7 Hz, 2H-12); ^{13}C NMR ($CDCl_3$): δ 16.10 (C-13), 16.74 (C-14 and C-15), 23.41 (C-8), 24.70 (C-2), 34.48 (C-3), 39.78 (C-11), 40.59 (C-9), 46.92 (C-7), 59.89 (C-4), 60.06 (C-10), 60.24 (C-1), 64.62 (C-12), 67.78 (C-5) and 69.44 (C-6); CIMS, *m/z* (%): 271 ($[M+1]^+$, 9), 253 ($[M+1-H_2O]^+$, 54), 235 ($[M+1-2xH_2O]^+$, 100), 217 ($[M+1-3xH_2O]^+$, 42).

Lactonization of product 10. Solid TPAP (tetrapropylammonium perruthenate, 7 mg) was added in a single portion to a stirred mixture of product **10** (34 mg), NMO (4-methylmorpholine N-oxide, 30 mg) and activated powdered molecular sieves (30 mg) in dry CH_2Cl_2 (5 ml) at room temperature under argon atmosphere. On completion, the reaction mixture was concentrated in a vacuum. Purification by column chromatography on silica gel yielded 29 mg of (1β,10α),(4β,5α)-diepoxy-7α,11β-H-germacran-6β,12-olide (**11**, 87%); White solid, mp 253-55°C; $[\alpha]_D$= -126° ($CHCl_3$, c 1); IR (KBr): 1768 and 1190 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.09 (1H, dd, $J_{9\alpha,9\beta}$= 14.4, $J_{9\alpha,8\beta}$= 12.0 Hz, H-9α), 1.22 (1H, ddd, $J_{3\alpha,3\beta}$= $J_{3\alpha,2\beta}$= 12.8, $J_{3\alpha,2\alpha}$= 8.4 Hz, H-3α), 1.30 (3H, s, 3H-14), 1.31 (3H, d, $J_{13,11}$= 7.6 Hz, 3H-13), 1.47 (3H, s, 3H-15), 1.55 (1H, dd, $J_{8\alpha,8\beta}$= 16.0, $J_{8\alpha,9\beta}$= 8.1 Hz, H-8α), 1.94 (1H, dd, $J_{7,8\beta}$= 9.1, $J_{7,6}$= 4.8 Hz, H-7), 2.06 (1H, ddd, $J_{8\beta,8\alpha}$= 16.0, $J_{8\beta,9\alpha}$= 12.0, $J_{8\beta,7}$= 9.1 Hz, H-8β), 2.18 (1H, dd, $J_{3\beta,3\alpha}$= 12.8, $J_{3\beta,2\beta}$= 7.4 Hz, H-3β), 2.36 (1H, dd, $J_{9\beta,9\alpha}$= 14.4, $J_{9\beta,8\alpha}$= 8.1 Hz, H-9β), 2.45 (1H, c, $J_{11,13}$= 7.6 Hz, H-11), 2.80 (1H, dd, $J_{1,2\beta}$= 9.4, $J_{1,2\alpha}$= 5.6 Hz, H-1), 2.93 (1H, d, $J_{5,6}$= 9.5 Hz, H-5) and 4.25 (1H, dd, $J_{6,5}$= 9.5, $J_{6,7}$= 4.8 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 15.46 (C-13), 16.42 (C-14), 17.05 (C-15), 24.48 (C-2), 26.54 (C-8), 33.94 (C-3), 41.12 (C-9), 47.63 (C-11), 48.43 (C-7), 59.05 (C-4), 59.90 (C-10), 60.45 (C-1), 61.27 (C-5), 79.24 (C-6) and 178.67 (C-12); HRLSIMS, *m/z*: $[M+1]^+$ 267.1593, ($C_{15}H_{23}O_4$ 267.1596, PPM 1.3).

Dehydration of product 4. To a stirred solution of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxy-11-hydroxy-germacrane (**4**, 100 mg) in CH_2Cl_2 (3 ml) we added Et_3N (0.2 ml) and DMAP (2 mg). The mixture was cooled to 0° C, and methanesulfonyl chloride (0.1 ml) was added dropwise. The mixture was stirred for 2 h at room temperature, then crushed ice was added and the mixture stirred for 1 h, after which it was extracted with CH_2Cl_2. The organic extracts were combined, washed with water, dried with anhydrous Na_2SO_4 and concentrated to dryness. Chromatography of the residue over silica gel yielded 64 mg (68%) of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxygermacr-11-ene (**12**); syrup; $[\alpha]_D$= -37° ($CHCl_3$, c 1); IR (film): 1736, 1236 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.33 (3H, s, 3H-15), 1.47 (3H, s, 3H-14), 1.72 (3H, bs, 3H-13), 2.04 (3H, s, AcO group), 2.92 (1H, d, J= 8.7 Hz, H-5), 3.03 (1H, dd, $J_{1,2\beta}$= 9.2, $J_{1,2\alpha}$= 6.1 Hz, H-1), 4.72 (1H, bs) and 4.81 (1H, m, w½= 3 Hz) (2H-12) and 4.92 (1H, dd, $J_{6,5}$= 8.7, $J_{6,7}$= 1.7 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 16.75 (C-14), 16.85 (C-15), 22.41 (C-13), 21.07 (*Me*CO), 23.05 (C-8), 24.66 (C-2), 34.25 (C-3), 40.33 (C-9), 49.12 (C-7), 59.49 (C-4), 59.84 (C-10), 60.24 (C-1), 65.31 (C-5), 73.02 (C-6), 112.15 (C-12), 146.12 (C-11) and 170.01 (Me*CO*); CIMS, *m/z* (%): 295 ($[M+1]^+$, 26), 235 ($[M+1\text{-}AcOH]^+$, 100), 217 (28), 199 (7).

Hydroboration of product 12. 64 mg of 6β-acetoxy-(1β,10α),(4β,5α)-diepoxygermacr-11-ene (**12**) were added to a solution (1 ml) of 9-BBN in THF (0.5 M). The mixture was stirred for 2 h at room temperature under argon atmosphere, then ethanol (0.6 ml), a 6 N solution of NaOH (0.2 ml) and H_2O_2 (30%) (0.4 ml) were added, and the mixture was heated for 1 h at 50°. Then the mixture was extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4 and concentrated to dryness. The residue was chromatographed over silica gel yielding 14 mg (24%) of the previously obtained product **10**, and 35 mg (60%) of (11R)-(1β,10α),(4β,5α)-diepoxy-6β,12-dihydroxygermacrane (**13**); syrup; $[\alpha]_D$= -28° ($CHCl_3$, c 1); IR (film): 3467 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3H, d, J= 7.1 Hz, 3H-13), 1.27 and 1.33 (3H each, s, 3H-14 and 3H-15) 2.83 (1H, d, J= 8.4 Hz, H-5), 2.95 (1H, dd, $J_{1,2\beta}$= 9.2, $J_{1,2\alpha}$= 5.8 Hz, H-1), 3.53 (1H, dd, $J_{6,5}$= 8.4, $J_{6,7}$= 1.3 Hz, H-6) and 3.42 and 3.57 (1H each, part AB of an ABX system, J_{AB}= 11.1, J_{AX}= 7.3, J_{BX}= 3.9 Hz, 2H-12); ^{13}C NMR ($CDCl_3$): δ 16.72 (C-14 and C-15), 16.90 (C-13), 19.38 (C-8), 24.70 (C-2), 34.49 (C-3), 41.22 (C-9), 41.33 (C-11), 45.44 (C-7), 59.93 (C-4), 60.06 (C-10), 60.37 (C-1), 64.18 (C-12), 67.87 (C-5) and 72.78 (C-6); CIMS, *m/z* (%): 271 ($[M+1]^+$, 11), 253 ($[M+1\text{-}H_2O]^+$, 59), 235 ($[M+1\text{-}2xH_2O]^+$, 100), 217 ($[M+1\text{-}3xH_2O]^+$, 38).

Lactonization of product 13. Solid TPAP (7 mg) was added in a single portion to a stirred mixture of product **13** (35 mg), NMO (30 mg) and activated powdered molecular sieves (30 mg) in dry CH_2Cl_2 (5 ml) at room temperature under argon atmosphere. On completion, the reaction mixture was concentrated to dryness. Chromatography on a silica gel column yielded 29 mg (84%) of (1β,10α),(4β,5α)-diepoxy-7α,11α-H-germacran-6β,12-olide (**14**); White solid, mp 257-59 °C; $[\alpha]_D$= -41° ($CHCl_3$, c 1); IR (KBr): 1775, 1182 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (3H, d, $J_{11,13}$= 7.6 Hz, 3H-13), 1.31 (3H, s, 3H-14), 1.48 (3H, s, 3H-15), 2.19 (1H, dd, $J_{3\beta,3\alpha}$= 12.8, $J_{3\beta,2\beta}$= 7.8 Hz, H-3β), 2.41 (1H, dd, $J_{9\beta,9\alpha}$= 14.4, $J_{9\beta,8\alpha}$= 7.9 Hz, H-9β), 2.84 (1H, dd, $J_{1,2\beta}$= 9.5, $J_{1,2\alpha}$= 5.4 Hz, H-1), 2.85 (1H, dc, $J_{11,7}$= $J_{11,13}$= 7.6 Hz, H-11), 2.93 (1H, d, $J_{5,6}$= 9.6 Hz, H-5) and 4.04 (1H, dd, $J_{6,5}$= 9.6, $J_{6,7}$= 4.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 9.95 (C-13), 16.42 (C-8), 16.46 (C-14), 17.13 (C-15), 24.55 (C-2), 34.03 (C-3), 41.33 (C-9), 41.62 (C-11), 46.36 (C-7), 59.10 (C-4), 59.21 (C-10), 60.34 (C-1), 61.39 (C-5), 80.26 (C-6) and 177.65 (C-12); HRLSIMS, *m/z*: $[M+1]^+$ 267.1598, ($C_{15}H_{23}O_4$ 267.1596, PPM -0.6).

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