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Enzyme-Catalyzed Rearrangement of a Diepoxy-germacrane Compound into New 7-*epi*-Eudesmane Derivatives

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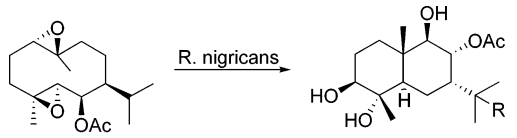
Received July 16, 2004



R = H or OH

Two new 7-*epi*-eudesmane derivatives, together with two new germacrane compounds, have been isolated from the microbial-transformation of a (1α,10*β*),(4*β*,5α)-diepoxygermacrane using the hydroxylating fungi *Rhizopus nigricans*. The rearranged skeleton and the stereochemistry of the chiral centers have been determined by means of their spectral data, and the absolute configuration has been confirmed by single-crystal X-ray analyses. A possible mechanism based on an enzyme-catalyzed isomerization to a 1α-hydroxy-(4*β*,5α)-epoxygermacr-9(*E*)-ene intermediate and a subsequent cyclization process is proposed in order to explain the formation of the 7-*epi*-eudesmane compounds.

Germacrane sesquiterpenes have received much attention in organic chemistry due to their central role in the biosynthesis of other sesquiterpene compounds.[1] In the most straightforward description, (*E*,*E*)-germacranes have been considered as direct precursors of both the *epi*- and the usual eudesmane sesquiterpenes.[2] *epi*-Eudesmanes, which possess an abnormal configuration at C-5 and/or C-10 or C-7,[3] have been isolated in plants, although not as much as the usual eudesmanes. Microbial reactions are significant tools in synthetic chemistry that enable the regio- and stereoselective functionalization of compounds, essential to preparing chiral building blocks and investigating their biosynthesis. Cytochrome P450-dependent oxygenases catalyze this enzymatic hydroxylation process and also play a fundamental role in the metabolism of terpenoids.[4]

In a previous work,[5] we described the microbial transformation of the (1*β*,10α)-epoxy derivative (**2**) of the natural germacrane **1** (shiromool acetate)[6] by a culture of *Rhizopus nigricans* CECT 2672, a synonym of *Rhizopus stolonifer* (ATCC 10404, IMI 061269).[7] This derivative was afforded by the stereoselective microbial epoxidation of the double bond of **1** and was also the main diastereomer isolated (59% yield) from the chemical epoxidation of **1** with *m*-chloroperbenzoic acid (MCPBA). Microbial transformation gave only hydroxylated germacrane derivatives, some of which allowed the semisynthesis of two new sesquiterpene lactones. The biotransformation of the minor epoxy derivative (**3**, 24% yield), achieved in the chemical epoxidation of **1**, by *R. nigricans* is now studied. The microorganism was cultured in a BEM medium[7] for 4 days, after which substrate **3** was added. Four new metabolites **4** (6%), **5** (10%), **6** (48%), and **7** (8%) were isolated after 7 days of incubation, together with some 10% of unaltered substrate (**3**) (Scheme 1).

Metabolites **4** and **5** showed the same molecular formula ($C_{17}H_{28}O_5$), suggesting the presence of an additional hydroxyl group in their respective molecules. The presence of two new signals centered at δ 3.83 and 3.81 as an AB system, in the ^{1}H NMR spectrum of **4**, together with the NOE effects observed between the protons of C-6 and those of C-15, the only singlet methyl signal, confirmed that compound **4** was a 14-hydroxyl derivative. On the other hand, the ^{1}H NMR spectrum of **5** revealed a signal at δ 3.70 due to the geminal proton of a hydroxyl group at C-2, this position was confirmed by the ^{13}C NMR data. The absolute stereochemistry at this carbon was determined by means of the Horeau method as "*R*";[8] therefore, **5** was 6*β*-acetoxy-(1α,10*β*),(4*β*,5α)-diepoxy-2*β*-hydroxygermacrane.

On comparing the NMR spectral data of metabolites **6** and **7** with those of compounds **3**, **4** and **5**, clear differences can be discerned, suggesting a possible skeleton rearrangement for the first ones. Furthermore, when the major metabolite (**6**, $C_{17}H_{30}O_5$) remained in a dichloromethane solution, a mixture of compounds was detected. The spectral data of the resultant mixture suggested the migration of the acetoxyl group that was confirmed, since the treatment of this mixture with Ac_2O−Py at room temperature afforded a single triacetylated compound (**8**, $C_{21}H_{34}O_7$). Therefore, the spectral data of stable derivative **8** (Scheme 2) were analyzed to determine the structure of metabolites **6** and **7**.

The analysis of the NMR data of compound **8** showed the absence of the epoxy group, a fact that could indicate a possible skeleton rearrangement. While five oxygenated carbons appeared in the 10-membered ring system of **3**,

(1) Cane, D. E. In *Comprehensive Natural Products Chemistry*; Cane, D. E., Ed.; Elsevier: London 1999; Vol. 2, pp 155−200.

(2) (a) Itokawa, H.; Morita, H.; Watanabe, K.; Mihashi, S.; Iitaka, Y. *Chem. Pharm. Bull*. **1985**, *33*, 1148−1153. (b) Marco, J. A.; Sanz-Cervera, J. F.; García-Liso, V.; Domingo, L. R.; Carda, M.; Rodríguez, S.; López-Ortíz, F.; Lex, J. *Liebigs Ann. Chem*. **1995**, 1837−1841.

(3) 7-*epi*-Eudesmanes are the antipodes of 5-*epi*-10-*epi*-eudesmanes and possess the *ent*-configuration.

(4) (a) Mihaliak, C.A.; Karp, F.; Croteau, R. *Methods Plant Biochem*. **1993**, *9*, 261−279. (b) Halkier, B. A. *Phytochemistry* **1996**, *43*, 1−21. (c) Nelson, D. R.; Koymans, L.; Kamataki, T.; Stegeman, J. J.; Feyereisen, R.; Waxman, D. J.; Waterman, M. R.; Gotoh, O.; Coon, M. J.; Estabrook, R. S.; Gunsalus, I. C.; Nebert, D. W. *Pharmacogenetics* **1996**, *6*, 1−42. (d) Azerad, R. In *Stereoselective Biocatalysis: Regio- and Stereoselective Microbial Hydroxylation of Terpenoids Compounds*; Patel, R. N., Ed.; Marcel Dekker, Inc.: New York 2000, pp 153−180.

(5) García-Granados, A.; Gutiérrez, M. C.; Martínez, A.; Rivas, F.; Arias, J. M. *Tetrahedron* **1998**, *54*, 3311−3320.

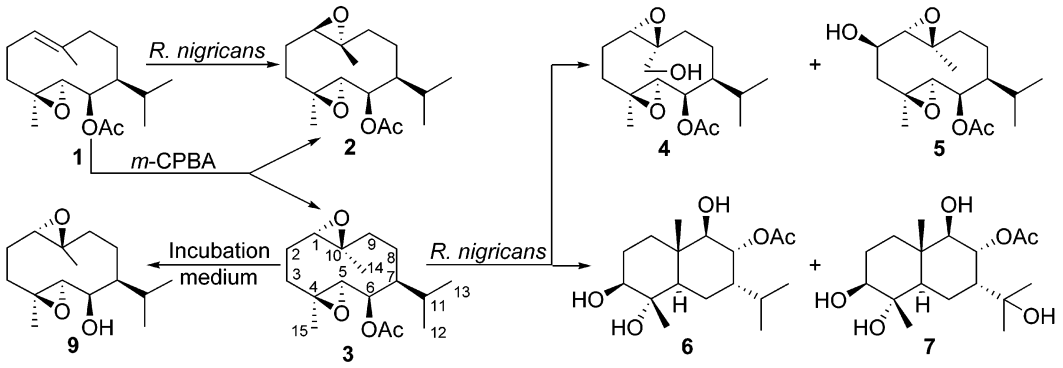
(6) García-Granados, A.; Molina, A.; Cabrera, E. *Tetrahedron* **1986**, *42*, 81−87.

(7) García-Granados, A.; Gutiérrez, M. C.; Rivas, F. *Org. Biomol. Chem.* **2003**, *1*, 2314−2320.

(8) Horeau, A. *Tetrahedron Lett*. **1961**, *12*, 506−512.

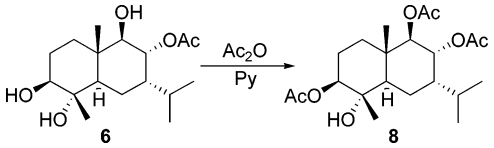
10.1021/jo048783k CCC: $30.25
Published on Web 12/03/2004

SCHEME 1. Synthesis of the Diepoxygermacranes 2 and 3[a]



[a] Biotransformation of **3** by *Rhizopus nigricans* cultures. Blank experiment conducted for **3**.

SCHEME 2. Acetylation of 6



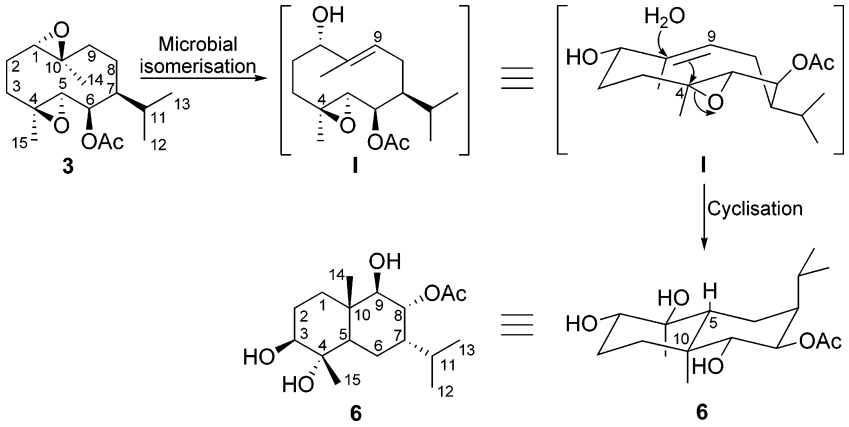
only four oxygenated carbons were found in the ring system of **8**, three oxymethines and an oxygen bearing quaternary carbon; i.e., three acetoxyl groups and a hydroxyl group were present in **8**. Furthermore, the presence of a new quaternary non-oxygenated carbon (δ_C 40.1) and also the characteristic signals for the isopropyl and for two methyl groups as singlet signals (^{1}H NMR spectrum) pointed toward a likely rearrangement to an eudesmane skeleton. The analysis of two-dimensional NMR experiments (^{1}H−^{1}H and ^{1}H−^{13}C COSY and HMBC experiments) agreed with this hypothesis. Carbons and protons on the rearranged skeleton were identified by the analysis of COSY experiments, while the HMBC experiment was used to place the functional groups in accordance with the corresponding numeration for the eudesmane skeleton. Hence, two acetoxyl groups were positioned on C-8 and C-9, since H-9 (δ_H 4.96) showed strong HMBC correlations to the new quaternary carbon (C-10), the methyl group situated on this carbon (C-14), and the oxygenated carbon C-8 (δ_H 5.07 δ_C 73.7), a fact that could explain the migration of the acetoxyl group detected before (between C-8 and C-9). The third acetoxyl group was situated on C-3, and the hydroxyl group was positioned on C-4, since these carbons showed a correlation to the protons of the methyl group at C-15 (δ_H 1.08), which also correlated to the methine C-5. The relative configuration of the chiral centers was determined by means of their proton-coupling constants and by the analysis of some NOE experiments. H-5 and the protons of the isopropyl group were spatially close, as were the protons situated at C-14 and C-15. Consequently, these results imply a 7-*epi*-eudesmane structure. These hypotheses and the absolute configuration of chiral carbons were confirmed by the X-ray data of derivative **8**, a 3β,8α,9β-triacetoxy-4α-hydroxy-7-*epi*-eudesmane. Subsequently, metabolite **6** was identified as 8α-acetoxy-3β,4α,9β-trihydroxy-7-*epi*-eudesmane and metabolite **7** was identified as its 11-hydroxy derivative in accordance with the β-effects detected on C-7, C-12, and C-13 (^{13}C NMR spectrum) and the presence of two deshielded methyl singlet signals (^{1}H NMR spectrum).

The stability of **3** toward the incubation medium was investigated as a blank experiment to test the possibility of nonenzymatic reactions. Thus, in absence of the hydroxylating fungus *R. nigricans*, some 90% of unaltered substrate (**3**) was recovered, while only 8% of the deacetylated derivative **9** was detected[9] (Scheme 1). Consequently, the transformation of the sesquiterpene skeleton must imply the action of the microorganism enzymes.

To explain the rearrangement, we propose a possible mechanism that suggests the isomerization of the (1α,-10β)-epoxy group of substrate **3** to a 1α-hydroxy-9*E*-ene derivative (**I**). This intermediate (**I**) would undergo a subsequent cyclization reaction to give **6** (Scheme 3). The blank experiment results, before described, led us to propose that the enzymatic system of the microorganism would be involved in this regioselective isomerization reaction. Although we have no evidence, a carbocationic pathway could be considered to better explain this isomerization process. Afterward, a transanular Markovnikov-type ring-opening of the epoxy group of the (9*E*)-ene intermediate (**I**), followed by the C-4/C-9 cyclization and subsequent stereoselective incorporation of a water molecule on C-10 would give **6**. The (*E*)-stereochemistry of the double bond of the intermediate **I** is crucial to explain the subsequent rearrangement to a 7-*epi*-eudesmane (**6**). Previous work has shown that the rearrangement of a similar compound with a cis stereochemistry in the double bond, the 4,5-epoxy of a (4*E*,9*Z*)-germacr-9-en-8-one, produced a 10-*epi*-eudesmane (*cis*-decaline).[10] The formation of **6** could be justified only if the cyclization occurred through a DD conformer of the supposed intermediate **I**.[11] In this case, an *all*-chair conformation, as previously described for 1(10),4-(*E*,*E*)-germacranes,[12] would not be favored due to the disadvantageous pseudo-axial position of the isopropyl group at C-7 versus the more favorable pseudo-equatorial

(9) Sanz, J. F.; Marco, J. A. *Phytochemistry* **1991**, *30*, 2788−2790.
(10) Piet, D. P.; Schrijvers, R.; Franssen, M. C. R.; de Groot, A. *Tetrahedron* **1995**, *51*, 6303−6314.
(11) Four different conformations of germacrene compounds are described in: Minnard, A. J.; Wijnberg, J. B. P. A.; de Groot, A. *Tetrahedron* **1999**, *55*, 2115−2146.
(12) Piet, D. P.; Minnaard, A. J.; van der Heyden, K. A.; Franssen, M. C. R.; Wijnberg, J. B. P. A.; de Groot, A. *Tetrahedron* **1995**, *51*, 243−254.

SCHEME 3. Proposed Mechanism for the Formation of 6



position displayed in a chair-boat conformation (Figure 1). Preliminary mechanic molecular studies would agree

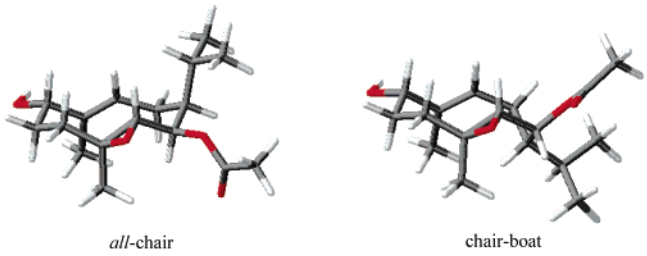


FIGURE 1. DD conformers for the intermediate **I**.

with this hypothesis since they showed the chair-boat conformation of **I** as the preferred one, with a *trans*-periplanar disposition of the bonds involved in the cyclization process. The stereoselective incorporation of the water molecule would take place from the β-face, less hindered in the DD conformer proposed, possibly by a concerted process, since a carbocation intermediate would not seem to be involved in the cyclization given that deprotonated metabolites coming from its neutralization were not isolated. As previous works have proposed, the transannular cyclization could be initiated by an enzyme-mediated action of the epoxy group.[10] This cyclization process would agree with the hypothesis of Hendrickson,[13] confirmed by de Groot,[14] which proposed a 9-ene germacrane derivative as a precursor of eudesmane compounds, although in this case, the presumed 1α-hydroxy-9*E*-ene derivative intermediate (**I**) leads exclusively to 7-*epi*-eudesmane derivatives as a consequence of the reduction in conformational space caused mainly by the epoxy groups that are unable to undergo the usual outside−inside rotation of a double bond.

Experimental Section

Organism, Media, and Culture Conditions. *Rhizopus nigricans* CECT 2672 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 BEM[7] medium containing 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 the corresponding microorganism. The cultures were incubated by shaking (150 rpm) at 28 °C for 4 days, after which substrate **3** (5−10%) in EtOH was added.

Biotransformation of 6β-Acetoxy-(1α,10β),(4β,5α)-diepoxigermacrane (3). Substrate **3** (240 mg) was dissolved in EtOH (3 mL), distributed among 5 Erlenmeyer flask cultures of *R. nigricans*, and incubated for 7 days, after which the cultures were filtered and pooled. The cells were washed thoroughly with water, and the liquid was saturated with NaCl and continuously extracted with CH_2Cl_2. Extracts were pooled, dried with anhydrous Na_2SO_4, and evaporated at reduced pressure to give a mixture of compounds. The mixture was chromatographed on a silica gel column to obtain the starting material **3** (25 mg, 10%), 6β-acetoxy-(1α,10β),(4β,5α)-diepoxi-14-hydroxygermacrane (**4**, 14 mg, 6%), 6β-acetoxy-(1α,10β),(4β,5α)-diepoxi-2β-hydroxygermacrane (**5**, 26 mg, 10%), 8α-acetoxy-3β,4α,9β-trihydroxy-7-*epi*-eudesmane (**6**, 123 mg, 48%), and 8α-acetoxy-3β,4α,9β,11-tetrahydroxy-7-*epi*-eudesmane (**7**, 22 mg, 8%).

6β-Acetoxy-(1α,10β),(4β,5α)-diepoxi-14-hydroxygermacrane (4): colorless solid, mp 109−111 °C; $[\alpha]^{25}_D$ +72° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 4.97 (1H, dd, $J_{6,5}$ = 7.1, $J_{6,7}$ = 1.8 Hz, H-6), 3.83 and 3.81 (1H each, AB system, $J_{A,B}$ = 11.9 Hz, 2H-14), 3.11 (1H, dd, $J_{1,2\alpha}$ = 10.8, $J_{1,2\beta}$ = 1.3 Hz, H-1), 3.01 (1H, d, $J_{5,6}$ = 7.1 Hz, H-5), 2.08 (3H, s, acetoxyl group), 1.30 (3H, s, 3H-15), 1.01 and 0.92 (3H each, d, J = 6.7 Hz, 3H-12 and 3H-13); ^{13}C NMR ($CDCl_3$) δ_C 170.2 (CH_3CO), 73.4 (C-6), 66.4 (C-5), 65.7 (C-14), 64.5 (C-10), 62.4 (C-1), 58.8 (C-4), 45.0 (C-7), 36.5 (C-3), 32.2 (C-9), 31.0 (C-11), 25.7 (C-2), 23.5 (C-8), 21.1 (CH_3-CO), 21.0 (C-12), 20.5 (C-13), 16.6 (C-15); IR ν_{max} (NaCl) 3477, 2928, 1736, 1238, 1024 cm^{-1}; HRLSIMS (*m/z*) 335.1839 ([M + 23]$^+$ $C_{17}H_{28}O_5Na$ requires 335.1834).

6β-Acetoxy-(1α,10β),(4β,5α)-diepoxi-2β-hydroxygermacrane (5): colorless syrup; $[\alpha]^{25}_D$ +49° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 4.95 (1H, dd, $J_{6,5}$ = 7.1, $J_{6,7}$ = 1.9 Hz, H-6), 3.70 (1H, ddd, $J_{2,3\beta}$ = 11.3, $J_{2,1}$ = 9.4, $J_{2,3\alpha}$ = 5.0, Hz, H-2), 3.07 (1H, d, $J_{5,6}$ = 7.1 Hz, H-5), 2.97 (1H, d, $J_{1,2}$ = 9.4 Hz, H-1), 2.54 (1H, dd, $J_{3\alpha,3\beta}$ = 13.0, $J_{3\alpha,2}$ = 5.0 Hz, H-3α), 2.07 (3H, s, acetoxyl group), 1.52 (3H, s, 3H-14), 1.31 (3H, s, 3H-15), 0.98 and 0.90 (3H each, d, J = 6.6 Hz, 3H-12 and 3H-13); ^{13}C NMR ($CDCl_3$) δ_C 170.2 (CH_3CO), 73.3 (C-6), 66.9 (C-1), 66.2 (C-5), 65.0 (C-2), 64.0 (C-10), 56.3 (C-4), 44.5 (C-3), 44.3 (C-7), 36.9 (C-9), 31.1 (C-11), 26.0 (C-8), 24.0 (C-14), 21.1 (CH_3CO), 20.9 (C-12), 20.7 (C-13), 18.2 (C-15); IR ν_{max} (NaCl) 3444, 2929, 1741, 1236, 1112 cm^{-1}; HRLSIMS (*m/z*) 335.1833 ([M + 23]$^+$ $C_{17}H_{28}O_5Na$ requires 335.1834). Determination of absolute configuration at C-2 (the Horeau method): 10 mg of metabolite **5** and 25 mg of racemic α-phenylbutyric anhydride (dissolved in 1 mL of pyridine),[8] $[\alpha]_D$ = +4° (*c* 2.5, $CHCl_3$).

(13) Hendrickson, J. B. *Tetrahedron* **1959**, *7*, 82−89.
(14) Minnaard, A. J.; Stork, G. A.; Wijnberg, J. B. P. A.; de Groot, A. *J. Org. Chem.* **1997**, *62*, 2344−2349.

8α-Acetoxy-3β,4α,9β-trihydroxy-7-*epi*-eudesmane (6): colorless syrup; ^{1}H NMR ($CDCl_3$) δ_H 4.87 (1H, dd, $J_{8,9}$ = 10.6, $J_{8,7}$ = 4.4 Hz, H-8), 3.44 (1H, d, $J_{9,8}$ = 10.6 Hz, H-9), 3.42 (1H, dd, $J_{3,2\beta}$ = 11.9, $J_{3,2\alpha}$ = 4.6 Hz, H-3), 2.06 (3H, s, acetoxyl group), 1.06 (3H, s, 3H-15), 0.97 and 0.90 (3H each, d, J = 5.8 Hz, 3H-12 and 3H-13), 0.91 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ_C 171.5 (CH_3CO), 79.3 (C-3), 77.4 (C-9), 77.1 (C-8), 75.5 (C-4), 45.8 (C-5), 42.3 (C-7), 40.6 (C-10), 35.7 (C-1), 27.2 (C-2), 26.2 (C-11), 24.0 (C-12), 22.0 (C-6), 21.9 (C-13), 21.5 (CH_3CO), 17.0 (C-15), 12.2 (C-14); IR ν_{max} (NaCl) 3448, 2933, 1716, 1253, 1079 cm^{-1}; HRLSIMS (*m/z*) 337.1989 ([M + 23]$^+$ $C_{17}H_{30}O_5Na$ requires 337.1991).

8α-Acetoxy-3β,4α,9β,11-tetrahydroxy-7-*epi*-eudesmane (7): colorless syrup; $[\alpha]^{25}_D$ −20° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 5.03 (1H, dd, $J_{8,9}$ = 9.7, $J_{8,7}$ = 6.3 Hz, H-8), 3.87 (1H, d, $J_{9,8}$ = 9.7 Hz, H-9), 3.46 (1H, dd, $J_{3,2\beta}$ = 11.9, $J_{3,2\alpha}$ = 4.4 Hz, H-3), 2.11 (3H, s, acetoxyl group), 1.33 and 1.32 (3H each, s, 3H-12 and 3H-13), 1.08 (3H, s, 3H-15), 0.92 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ_C 171.4 (CH_3CO), 79.4 (C-3), 78.5 (C-9), 77.4 (C-8), 75.9 (C-4), 73.9 (C-11), 45.7 (C-5), 44.1 (C-7), 40.4 (C-10), 36.4 (C-1), 32.6 (C-12), 30.5 (C-13), 27.3 (C-2), 21.3 (C-6), 21.7 (CH_3CO), 16.6 (C-15), 12.0 (C-14); IR ν_{max} (NaCl) 3433, 2932, 1717, 1256, 1035 cm^{-1}; HRLSIMS (*m/z*) 353.1948 ([M + 23]$^+$ $C_{17}H_{30}O_6Na$ requires 353.1940).

Acetylation of 6. Metabolite **6** (50 mg) was dissolved in pyridine (2 mL) and Ac_2O (1 mL). The mixture was stirred for 7 h at room temperature and extracted in the usual way. Purification by column chromatography on silica gel yielded 54 mg (85%) of 3β,8α,9β-triacetoxy-4α-hydroxy-7-*epi*-eudesmane (**8**), a colorless solid: mp 78−80 °C; $[\alpha]^{25}_D$ −23 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 5.07 (1H, dd, $J_{8,9}$ = 11.1, $J_{8,7}$ = 5.1 Hz, H-8), 4.96 (1H, d, $J_{9,8}$ = 11.1 Hz, H-9), 4.64 (1H, dd, $J_{3,2\beta}$ = 11.6, $J_{3,2\alpha}$ = 4.8 Hz, H-3), 2.07 (1H, br d, $J_{6\alpha,6\beta}$ = 13.0 Hz, H-6α), 2.06, 2.00 and 1.96 (3H each, s, acetoxyl groups), 1.53 (1H, br d, $J_{5,6\beta}$ = 13.0 Hz, H-5), 1.44 (1H, ddd, $J_{6\beta,5} = J_{6\beta,6\alpha}$ = 13.0, $J_{6\beta,7}$ = 3.7 Hz, H-6β), 1.37 (1H, ddd, $J_{1\alpha,2\beta} = J_{1\alpha,1\beta}$ = 13.7, $J_{1\alpha,2\alpha}$ = 3.5 Hz, H-1α), 1.08 (3H, s, 3H-15), 0.99 (3H, s, 3H-14), 0.98 and 0.93 (3H each, d, J = 6.4 Hz, 3H-12 and 3H-13); ^{13}C NMR ($CDCl_3$) δ_C 171.6 (CH_3CO), 170.5 (CH_3CO), 170.4 (CH_3CO), 81.2 (C-3), 77.5 (C-9), 73.9 (C-4), 73.7 (C-8), 46.5 (C-5), 42.3 (C-7), 40.1 (C-10), 35.0 (C-1), 26.1 (C-11), 24.8 (C-2), 24.1 (C-12), 21.8 (C-6), 21.7 (C-13), 21.3 (CH_3CO), 21.2 (CH_3CO), 20.9 (CH_3CO), 17.7 (C-15), 13.2 (C-14); HMBC (H → C) H-5 → C-4, 6, 10, 14, 15; H-9 → C-1, 8, 10, 14; 3H-12 and 3H-13 → C-7, 11; 3H-14 → C-1, 5, 9, 10; 3H-15 → C-3, 4, 5; IR ν_{max} (NaCl) 3500, 2955, 1740, 1246, 1112 cm^{-1}; HRLSIMS (*m/z*) 421.2205 ([M + 23]$^+$ $C_{21}H_{34}O_7Na$ requires 421.2202).

Blank Experiment Incubation. Substrate **3** (80 mg) was dissolved in EtOH (1 mL), added to 1 Erlenmeyer flask with 75 mL of sterile BEM[7] medium, and incubated for 7 days under the same conditions of the biotransformation of substrate **3**. Then, the mixture was processed as indicated above to give the unaltered substrate **3** (72 mg, 90%) and 6β-hydroxy-(1α,10β),-(4β,5α)-diepoxigermacrane[9] (**9**, 6 mg, 8%).

Acknowledgment. This work was financially supported by grants from the Comisión Interministerial de Ciencia y Tecnología (PM98-0213), the Consejería de Educación y Ciencia de la Junta de Andalucía (FQM-0139), and a predoctoral fellowship from the Ministerio de Educación Cultura y Deporte. We thank David Nesbitt for reviewing the English in the manuscript. We would like to thank José Romero for reviewing the CIF files and José A. Dobado for the preliminary theoretical conformational studies.

Supporting Information Available: "General Experimental Methods" and copies of ^{1}H and ^{13}C NMR spectra of compounds **4**−**8**, NOE spectra of compounds **4** and **8**, and ^{1}H−^{1}H and ^{1}H−^{13}C COSY and HMBC spectra and crystal data (CIF file) for compound **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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