Regioselective Enzymatic Acylations of Polyhydroxylated Eudesmanes: Semisynthesis, Theoretical Calculations, and Biotransformation of Cyclic Sulfites

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Different lipase enzymes have been tested in order to perform regioselective acetylations on the eudesmane tetrol from vulgarin. High yields (95%) of 1,12-diacetoxy derivative (**4**) were achieved in 1 h with *Candida antarctica* lipase (CAL). However, only the 12-acetyl derivative (**6**) was obtained in similar yield with *Mucor miehei* (MML) or *Candida cylindracea* (CCL) lipases. The enzymatic protection at C-1 and C-12 has been used to form eudesmane cyclic-sulfites between C-6 and C-4 atoms. The *R*/*S*-sulfur configuration has been assigned by means of the experimental and theoretical ^{13}C and ^{1}H NMR chemical shifts. The theoretical shifts were calculated using the GIAO method, with a MM+ geometry optimization followed by a single-point calculation at the B3LYP/6-31G* level (B3LYP/6-31G*//MM+). Moreover, B3LYP/6-31G* geometry optimizations were carried out to test the B3LYP/6-31G*//MM+ results, for the deacetylated sulfites (**12** and **15**). In addition to the δ_C and δ_H shifts, the $^3J_{HH}$ coupling constants were also calculated and compared with the experimental values when available. Finally, different reactivities have been checked in both sulfites by biotransformation with *Rhizopus nigricans*. While the *R*-sulfite gave 2α- and 11*β*-hydroxylated metabolites, the *S*-sulfite yielded only regioselective deacetylations. Furthermore, both sulfites showed different reactivities in redox processes.

I. Introduction

Sesquiterpene compounds with eudesmane skeletons are widespread in nature[1,2] and are used frequently in organic synthesis.[3−7] In recent years, the search for novel and highly selective reactions has increased, and among the new methods to achieve this selectivity is the biotransformation using microorganisms[8−11] and enzymes.[12,13] In this context, lipases are the most common enzymes for a selective hydroxyl group protection,[14−16] enabling the chemical and biological semisyntheses of valuable natural products. In previous works,[11,17] our group has employed lipases for semisynthetic purposes. Thus, the α-santonin hexahydro derivatives were sequentially isolated with the assistance of enzymatic selective acylations.[17] Furthermore, in the same work, a semisynthesis of 8,12-eudesmanolides was performed using regioselective deacetylations with lipases. On the other hand, with combined chemical and microbiological methods, *enantio*-3-hydroxyambrox derivatives are semisynthesized from *ent*-12-oxo-13-*epi*-manoyl oxides.[11] In this semisynthesis, a regioselective acetylation is carried out from *ent*-3,12-dihydroxy-13-*epi* manoyl oxide with

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Table 1. Products and Yields at Different Reaction Times for Enzymatic Acetylations of 3

enzymes	1 h product (%)	8 h product (%)	24 h product (%)	48 h product (%)	96 h product (%)	168 h product (%)
CAL[a]	**4** (95)	**4** (95)	**4** (95)	**4** (95)	**4** (95)	**4** (95)
MML[a]	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (10); **6** (90)	**4** (20); **6** (70); **5** (10)	**4** (20); **6** (70); **5** (10)
CCL[b]	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (10); **6** (90)	**4** (20); **6** (80)	**4** (20); **6** (80)
PPL[b]	**4** (5); **6** (15); **7** (10)	**4** (5); **6** (20); **7** (10)	**4** (10); **6** (35); **7** (10)	**4** (10); **6** (40); **7** (15)	**4** (15); **6** (45); **7** (15)	**4** (25); **6** (55); **7** (15)
Lipase A[c]	**6** (60)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)
Lipase AYS[c]	**6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)	**4** (5); **6** (95)
Newlase F[c]		**6** (5)	**6** (5)	**6** (5)	**6** (10)	**6** (10)
Lipase AK20[c]	**6** (90)	**4** (10); **6** (90)	**4** (15); **6** (85)	**4** (65); **6** (35)	**4** (80); **6** (20)	**4** (95); **6** (5)
Lipase PS[c]	**6** (75)	**4** (10); **6** (90)	**4** (10); **6** (90)	**4** (60); **6** (40)	**4** (65); **6** (35)	**4** (70); **6** (30)

[a] Available from Novo-Nordisk. [b] Available from Aldrich. [c] Available from Amano.

Candida cylindracea (CCL) and *Candida antarctica* (CAL) lipases. Selective chemical acetylations have been also performed on similar substrates, enabling epimerization at C-6 the 6α-eudesmanolides and in turn yielding the scarce natural products 6*β*-eudesmanolides.[18]

The synthetic interest of isolated enzymes or microorganisms in organic chemistry are in the regio- and stereoselectivity of both enzymatic systems. With these enzymatic pathways, particular functional groups can be transformed without any change in other parts of the substrate or access becomes possible to positions that are problematic by normal chemical processes. In terms of semisynthesizing natural products, the generation of a new hydroxyl group in the molecule is very useful. This versatile hydroxyl can be easily transformed to open new series of possible reactions at the corresponding carbons and in the neighboring region.

On the other hand, stereoselective transformations of diols have recently been developed via cyclic sulfite intermediates.[19−21] These sulfites can yield selective ring opening with strong nucleophiles, giving sulfur dioxide and a suitable stereoisomer. Due to the difficulties in the NMR structural study for the stereoisomeric mixture, its resolution is determined mainly by X-ray crystallography or by its oxidation to sulfates, which are suitable to nucleophilic attacks.[22,23]

Accurate geometries are easily available for small- to medium-sized molecules by theoretical calculations of considerable quality, such as DFT method with double-*ζ* split-valence basis sets augmented with polarization functions.[24] However, the quality of the Molecular Mechanics (MM+)[25] geometries should be tested by adequate MO calculations.

These theoretical methods can also be used to calculate ^{13}C and ^{1}H NMR chemical shifts using the gauge including atomic orbitals (GIAO) method,[26] yielding data comparable to those of the experiment.[27] The time-consuming DFT geometries can be replaced by the computationally inexpensive MM+ ones, making it possible to calculate the ^{13}C and ^{1}H shifts in large systems. Moreover, the density functional theory, with the B3LYP hybrid functional that includes electron correlation, has proven accurate to predict these shifts.[27] From the theoretical geometries accurate $^{3}J_{HH}$ coupling constants are also available, applying the equation of Haasnoot−Leeuw−Altona,[28] yielding useful structural information (conformational and configurational) for the triterpene derivatives.

In the present paper, the different activities of lipase enzymes as acylating agents have been studied for the protection of particular hydroxyl groups, on polyhydroxyeudesmane from natural sources. With the use of an adequate functionalized product, a diastereomer sulfite mixture was formed between the C-4 and C-6 positions of the eudesmane skeleton. Moreover, a structural study of the sulfite pair of compounds has been performed using theoretical and experimental NMR chemical shifts and the $^{3}J_{HH}$ coupling constants. Finally, a reactivity study has been made of both diastereomer sulfites by redox processes and biotransformations with the *Rhizopus nigricans*.

II. Results and Discussion

(a) Semisynthesis. Vulgarin (**1**) is a 6α-sesquiterpene lactone widely abundant and isolated from *Artemisia canariensis*.[18,29] Hydrogenation of vulgarin (**1**) yielded the tetrahydro derivative (**2**, 98%) (1*β*-hydroxycolartin)[18] and further reaction of **2** with $LiAlH_4$/THF gave the tetrahydroxy derivative (**3**, 85%). This tetrol with Ac_2O/Py, under controlled conditions, generated the 1,12-diacetoxy (**4**) and 1,6,12-triacetoxy (**5**) derivatives with satisfactory regioselectivities.[18] However, a selective acetylation with synthetic purposes was better performed via enzymatic pathways using lipases. To determine the optimal conditions for the enzymatic acetylation of **3**, the Novo-Nordisk lipases *C. antarctica* (CAL) and *Mucor Miehei* (MML); the Aldrich lipases *C. cylindracea* (CCL) and *Porcine pancreas* (PPL); and the Amano Lipase A, Lipase AYS, Newlase F, Lipase AK, and Lipase PS were used as biocatalysts. Moreover, vinyl acetate (VA) was employed as a solvent and acetylating agent, and the enzyme: substrate ratio was fixed at 6:1. The results with different reaction times are summarized in Table 1, showing that

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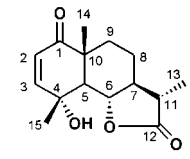

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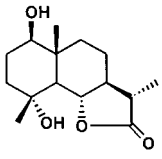

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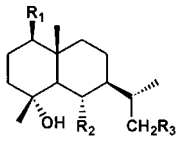


Product	R_1	R_2	R_3
3	OH	OH	OH
4	OAc	OH	OAc
5	OAc	OAc	OAc
6	OH	OH	OAc
7	OAc	OH	OH

Figure 1. Structures of compounds **1**−**7**.

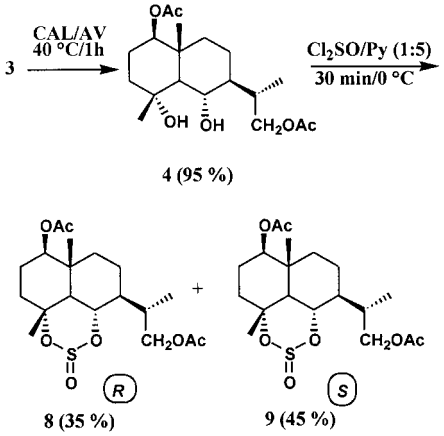
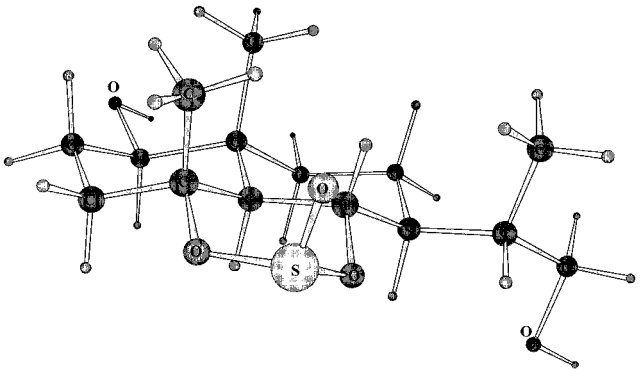


Figure 2. Semisynthesis of *R*/*S*-sulfites (**8** with an axial S → O bond and *R* configuration on sulfur atom and **9** with an equatorial S → O bond and *S* configuration on sulfur atom).

the acetylation with CAL on C-1 and C-12 took place with high selectivity, yielding only **4,** regardless of the reaction time. Furthermore, MML, CCL, Lipase A, and Lipase AYS gave high yields for the monoacetate **6,** confirming the noticeable selectivity of these enzymes at the C-12 position. However, with MML the amount of monoacetate **6** diminished with the reaction time, giving rise to diacetate **4** and even a small amount of triacetate **5**. On the other hand, PPL was less active, yielding variable amounts of **4** and **6** (invariably as main products) with the reaction time, and with a small amount of monoacetate (**7**) at C-1. Finally, it was noteworthy that the Lipases AK and PS also yielded **4** and **6** but with less regioselectivity and that Newlase F was inactive, giving only an insignificant amount of **6** (see Figure 1).

One of the most notable results from the assays performed was that CAL gave **4** with high regioselectivity (95%), with the positions 1 and 12 protected, and enough yield for the semisynthesis of other derivatives. Tetrol **3** reacted with vinyl VA and CAL (6:1) for 1 h. Then, **4** was treated with thionyl chloride in pyridine (1:5) for 30 min at 0 °C, yielding the cyclic sulfites (both with α orientation) between the free hydroxyl groups located at C-4 and C-6. Thus, a diastereomeric mixture of cyclic sulfites in different ratios appeared with **8** (35%) and **9** (45%) (see Figure 2). The two products presented different chemical and physical properties (polarity, α_D, reactivity); however, in the mass spectra both had a molecular ion peak M^+ with $m/z = 402$ ($C_{19}H_{30}O_7S$).

Nevertheless, the two diasteromer sulfites **8** and **9** differed in several ^1H and ^{13}C NMR chemical shifts (with



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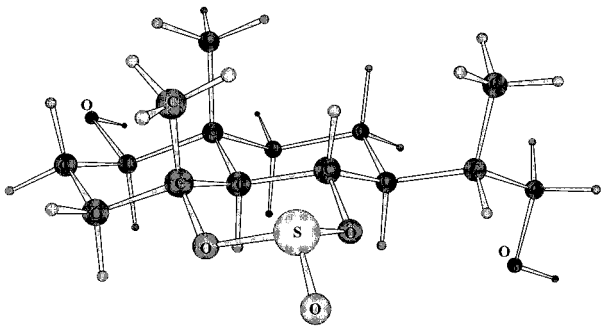

Figure 3. Representation of the deacetylated geometries (**12** with an axial S → O bond and *R* configuration on sulfur atom and **15** with an equatorial S → O bond and *S* configuration on sulfur atom) obtained at the B3LYP/6-31G*//B3LYP/6-31G* theoretical level.

different δ_H values for H-6β and 3H-15). In **8**, the H-6β was at 4.84 ppm (1H, dd, $J_1 = J_2$ 10.3 Hz) while in **9** this proton appeared at 4.43 ppm with similar coupling constants. Similarly, the C-15 methyl group was at 1.81 (3H, s) in **8** and at 1.65 ppm (3H, s) in **9**. Given the ^{13}C NMR spectra of this pair of compounds, the most noticeable differences were between C-6 (66.1 for **8** and 74.7 ppm for **9**) and C-15 (25.4 for **8** and 22.7 ppm for **9**). These data together with the theoretical structures for **8** and **9** enabled us to establish how the S → O was situated in the sulfite cycle and consequently the absolute sulfur configuration in each compound (see Figure 3). Considering the high *trans*-decalin rigidity in the eudesmane skeleton and the new sulfite cycle between 4α and 6α positions in both isomers (**8** with an axial S → O bond and R configuration on the sulfur atom, and **9** with an equatorial S → O bond and S configuration on the sulfur), the sulfite cycle had an almost identical chair conformation. However, in compound **8**, the S → O bond was in a syn-diaxial position with the C-4 methyl group (C-15) and with H-6β, while this bond should form 120° with these substituents in compound **9**, compatible with the theoretical puckering geometrical parameters listed in Table 2. This S → O orientation explained the large deshielding in the H-6β and H-15 signals for **8**, in which there was a 1,3-diaxial position with the oxygen atom nearest these hydrogens. There was also a noticeable difference in the δ_C values for **8** and **9**. Thus, C-6 was situated at a higher field (66.1 ppm) than in **9** (74.7 ppm). This difference can be explained by the fact that **8** had γ-gauche disposition between the S → O bond and C-6 (with the corresponding shielding effect on this carbon). A similar and smaller γ-gauche effect was also appreciated on C-4 (81.6 for **8**

Table 2. Selected[a] Geometrical Parameters (Å and Deg) and Puckering[b] Values for the Three Six-Membered Rings (*Q* in Å, and Θ and Φ in Deg)

	8 *R* (ax)	**9** *S* (eq)	**12** *R* (ax)	**15** *S* (eq)	**17** *R* (ax)	**14** *S* (eq)	**18** *R* (ax)	**13** *S* (eq)
C_4-O_1	1.417	1.418	1.418 (1.480)	1.418 (1.469)	1.418	1.419	1.417	1.419
O_1-S	1.676	1.683	1.675 (1.666)	1.682 (1.679)	1.674	1.683	1.674	1.683
$S-O_3$	1.465	1.481	1.466 (1.477)	1.481 (1.463)	1.466	1.481	1.466	1.481
O_2-S	1.672	1.680	1.671 (1.664)	1.679 (1.678)	1.671	1.680	1.672	1.679
C_6-O_2	1.412	1.414	1.413 (1.463)	1.414 (1.458)	1.413	1.413	1.413	1.413
$\angle C_4O_1S$	118.6	112.0	118.7 (120.0)	112.0 (115.3)	118.6	111.9	118.7	112.0
$\angle O_1SO$	101.5	91.9	101.5 (109.9)	91.8 (104.6)	101.5	91.8	101.6	91.8
$\angle O_2SO_1$	100.2	94.6	100.3 (98.1)	94.5 (97.3)	100.3	94.6	100.2	112.0
$\angle O_2SO$	101.5	91.7	99.0 (107.7)	92.0 (104.8)	99.0	92.0	98.8	91.7
$\angle SO_2C_6$	117.0	112.0	117.0 (117.7)	111.8 (116.0)	117.0	111.8	117.0	112.0
$\angle C_4O_1SO$	51.5	−160.2	51.8 (59.2)	−160.8 (−168.3)	51.7	−160.7	51.5	−160.2
$\angle C_6O_2SO$	−52.1	159.1	−52.1 (−59.5)	159.4 (165.4)	−52.1	159.4	−52.1	159.2
Q_A	0.565	0.559	0.563 (0.579)	0.559 (0.570)	0.564	0.560	0.564	0.559
Θ_A	3.7	2.4	3.7 (3.5)	2.6 (2.7)	3.6	2.5	3.8	2.4
Φ_A	233.8	217.2	231.8 (226.0)	212.4 (257.1)		209.8	214.4	233.5
Q_B	0.560	0.565	0.559 (0.561)	0.564 (0.564)		0.564	0.564	0.560
Θ_B	6.7	4.6	6.8 (6.4)	4.8 (4.6)		4.7	4.9	6.8
Φ_B	237.3	229.6	237.7 (245.3)	231.7 (256.3)		232.7	230.8	236.9
Q_C	0.566	0.685	0.565 (0.589)	0.688 (0.630)		0.686	0.688	0.565
Θ_C	3.6	12.9	3.7 (2.6)	13.1 (6.1)		12.9	13.0	3.6
Φ_C	101.7	244.0	103.7 (143.3)	243.7 (267.2)		243.7	243.4	101.6

[a] MM+ values and in parentheses the B3LYP/6-31G* ones. [b] Ring A formed by $C_1-C_2-C_3-C_4-C_5-C_{10}$, ring B by $C_5-C_6-C_7-C_8-C_9-C_{10}$, and ring C by $C_4-C_5-C_6-O_1-S-O_2$.

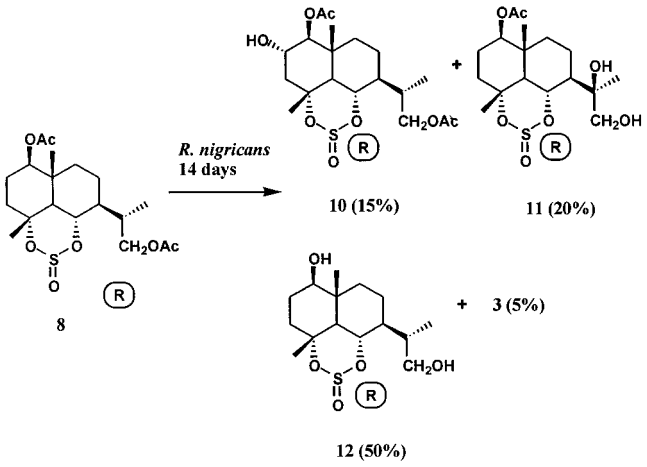

Figure 4. Biotransformation of *R*-sulfite **8** with *Rhizopus nigricans*.

and 82.6 ppm for **9**). Based on these observations and the theoretical data, we can give the structure of 1β,12-diacetoxy-5α,11β-H-eudesman-4α,6α-diyl-*S*(*R*)-cyclic sulfite for **8** and 1β,12-diacetoxy-5α,11β-H-eudesman-4α,6α-diyl-*S*(*R*)-cyclic sulfite for **9**.

To discern the two possible estereomeric sulfites (**8** and **9**) by their reactivity, we performed three different reactions, a biotransformation with the fungus *Rhizopus nigricans*, a regioselective chemical deacetylation with $NaBH_4$, and a convergent oxidative process with $RuCl_3$/$NaIO_4$. The biotransformation of **8**, (*R*-configuration on sulfur atom), with *R. nigricans* for 14 days gave **10** (15%), **11** (20%), **12** (50%), and **3** (5%) (Figure 4). However, its isomer **9** (*S*-configuration on sulfur atom), under identical conditions, yielded **13** (15%), **14** (10%), **15** (60%), **16** (5%), and **3** (5%) (Figure 5). Comparing the isolated compounds in both biotransformations, we found different behaviors of the two sulfites in their incubations with *R. nigricans*, although the main products from these biotransformations were the C-1 and C-12 deacetylated compounds by the fungus (**12** and **15**). However, two metabolites appeared for the *R*-isomer (**8**) with new hydroxylations at C-2α (**10**) and C-11β (**11** deacetylated also at C-12) of the eudesmane skeleton. Incubation of **9** (*S*-isomer) gave two regioselectively deacetylated products at C-1 and C-12 (**14** and **13**), but there were no new hydroxylated compounds for this microbiological process. Moreover, from the biotransformation of **9**, a minor product (**16**) was isolated as a result of the sulfite cycle opening at C-4 by the microorganism, giving a C-4/C-15 exocyclic double bond and leaving an α-sulfinyloxy group at C-6. Finally, in both cases small amounts of **3** were obtained.

Subsequently, compounds **8** and **9** were treated with $NaBH_4$ at room temperature while controlling the percentage of deacetylation with the reaction time (Figure 6). The results are summarized in Table 3, and again sulfite **8** was most reactive, giving the highest percentage of deacetylation. The S → O axial position in **8** can assist in the deacetylation process and, therefore, two new products, **17** and **18**, were obtained. The 12- and 1-deacetylated derivatives (**17** and **18**, respectively) were not produced in the biotransformation of *R*-sulfite (**8**) with the above-mentioned fungus. On the other hand, the *S*-sulfite (**9**) led to the totally or partially deacetylated compounds (**13**, **14**, and **15**), although in lower yields.

The above reactions allow us to protect enzymatically and with high selectivity some positions in the sesquiterpene skeleton, to perform further reactions on other positions and by combining chemical with microbiological methods to semisynthesize both R/S sulfites **8** and **9**. Moreover, by means of a biotransformation of **8** with *R. nigricans*, we obtained remote hydroxylated metabolites at C-2 and C-11 with acceptable large yields, the semisynthesis of these metabolites being extremely difficult to achieve by classical chemical reactions.

Finally, **8** and **9** were individually and completely oxidized to a cyclic sulfate **19** by a convergent oxidative process using $RuCl_3/NaIO_4$ (see Figure 6). The most remarkable aspect of this process was that, while **8** needed 12 h for its total conversion, **9** required only 3 h. Therefore, in the oxidative process to the corresponding cyclic sulfate, the equatorial S → O sulfite was signifi-

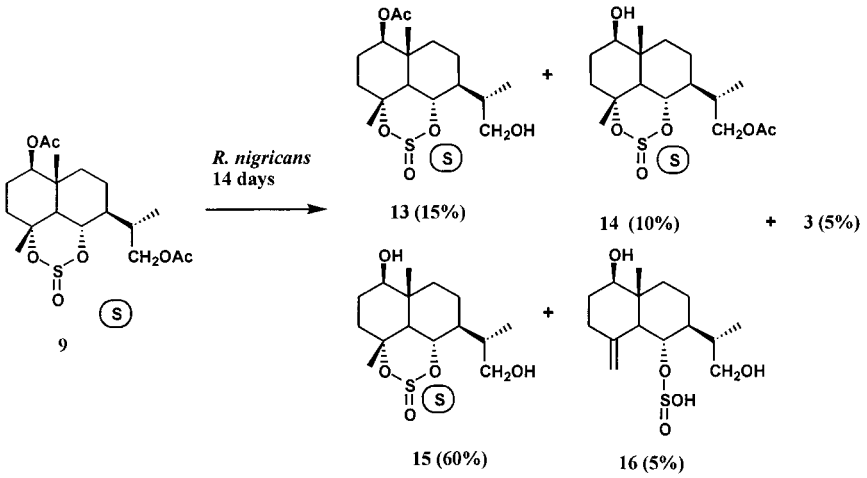

Figure 5. Biotransformation of *S*-sulfite **9** with *Rhizopus nigricans*.

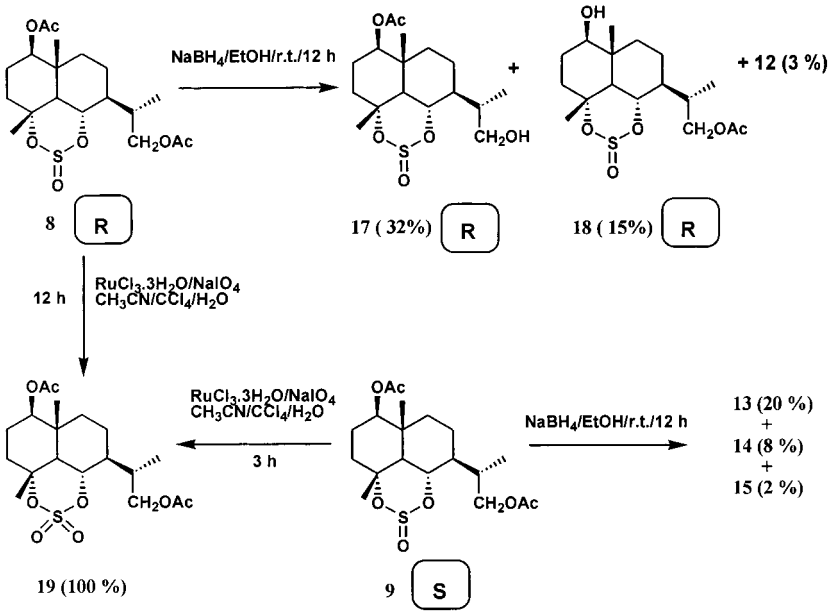

Figure 6. Redox reactions of diastereomeric sulfites **8** and **9**.

Table 3. Deacetylation Results in the Treatment of Diastereomeric Sulfites (8 and 9) with Sodium Borohydride

reaction time (h)	sulfite **8** product (%)			sulfite **9** product (%)		
6	**17** (19)	**18** (9)	**12** (2)	**13** (6)	**14** (3)	**15** (1)
12	**17** (32)	**18** (15)	**12** (3)	**13** (20)	**14** (8)	**15** (2)
24	**17** (45)	**18** (25)	**12** (5)	**13** (40)	**14** (20)	**15** (4)

cantly more reactive than the axial one. The new cyclic sulfate **19** had a molecular ion peak with (m/z = 418) 16 units of m/z more than the related sulfites, and its ^{1}H NMR spectrum was quite similar to that of sulfite **8** (axial), since **19** had two sulfur exocyclic oxygen atoms, with one also approximately in this position. Consequently, the H-6β and 3H-15 in this compound were also in this compound quite deshielded (4.77 for H-6 and 1.77 ppm for 3H-15). The ^{13}C NMR spectrum confirmed the structure of **19** although the C-6 and C-4 shifts were larger that in **8** (with no γ-gauche effect of the C=O bond).

(b) Stereochemistry and Theoretical Calculations. The stereochemistry and structure of the above-mentioned *R*/*S*-sulfite derivatives explain their theoretical characterization. These compounds are specified by their sulfur configuration (*R* for **8**, **12**, **17**, and **18**; and *S* for **9**, **13**, **14**, and **15**). The structures with an *R*-configuration had an axial arrangement of the oxygen bonded to the S atom, while the *S*-sulfite had an equatorial position. Compounds **12** and **15** had the hydroxyl groups at C-1 and C-12 positions, and the remaining compounds presented different acetylations at these positions, **8** and **9** being the corresponding diacetylated derivatives. To simplify the theoretical calculations, we used the rigorous B3LYP/6-31G* method on the deacetylated derivatives (**12** and **15**). Both compounds were fully optimized in this DFT method (Figure 3). Moreover, these geometries were compared with their corresponding MM+ ones, and the agreement between the very expensive time-consuming DFT and the easily available MM+ methods was remarkable (see Table 2). In addition, the electronic properties found with the B3LYP/6-31G*//MM+ scheme yielded accurate results for the δ_C chemical shifts.[27]

Taking into account the lack of experimental geometrical information (X-ray data) for these compounds,[30]

Table 4. Experimental and Calculated (at the B3LYP/6-31G*//MM+ Theoretical Level) NMR ^{13}C Chemical Shifts

	8 *R* (axial)		**9** *S* (equatorial)		**12** *R* (axial)			**15** *S* (equatorial)			**17** *R* (axial)		**14** *S* (equatorial)		**18** *R* (axial)		**13** *S* (equatorial)	
δ_C	exptl	calcd	exptl	calcd	exptl	calcd[a]	calcd	exptl	calcd[a]	calcd	exptl	calcd	exptl	calcd	exptl	calcd	exptl	calcd
1	79.3	72.8	79.2	72.8	78.4	79.2	73.2	78.3	79.0	73.2	79.4	73.2	78.3	73.2	78.4	73.0	79.3	72.9
2	23.9	24.6	24.3	25.1	27.4	27.2	26.1	27.7	27.4	26.8	23.9	26.1	27.8	26.8	27.4	24.4	24.3	25.1
3	38.6	39.8	38.4	37.7	38.9	39.9	40.3	38.6	39.1	38.2	38.6	40.2	38.7	38.2	38.9	39.8	38.3	37.8
4	81.6	66.4	82.6	65.2	82.2	80.0	66.7	83.4	79.1	65.5	81.7	66.7	83.1	65.4	82.0	66.3	82.8	65.1
5	52.9	47.1	50.3	48.5	52.9	52.8	46.8	50.5	51.4	48.2	53.0	46.8	50.2	48.5	52.8	46.9	50.5	48.2
6	66.1	53.0	74.7	60.1	67.1	63.6	53.2	75.6	71.2	60.2	66.7	53.1	75.1	60.2	66.5	52.9	75.1	60.1
7	43.1	40.4	44.6	41.1	42.6	41.1	39.1	44.3	41.9	39.9	42.5	40.4	44.9	41.1	43.3	39.1	44.4	39.9
8	18.1	17.9	18.9	18.1	18.7	18.9	18.4	19.1	19.2	18.5	18.5	17.9	19.2	18.3	18.4	18.4	19.2	18.5
9	39.3	40.4	39.1	40.6	39.5	40.8	39.9	39.4	40.3	40.1	39.3	39.9	39.3	40.1	39.5	40.5	39.2	40.7
10	38.3	35.5	38.9	35.7	39.3	41.8	36.7	39.9	41.9	36.8	38.3	36.8	39.9	36.9	39.3	35.6	38.9	35.6
11	30.5	29.5	30.3	29.4	34.4	33.3	31.2	33.8	32.8	31.0	34.4	29.4	30.3	29.3	30.6	31.4	34.0	31.0
12	67.2	60.9	67.1	60.7	66.0	64.7	61.5	66.1	64.7	61.4	66.0	60.9	67.3	60.7	67.4	61.5	66.3	61.5
13	11.4	11.2	11.3	11.5	11.7	11.4	12.0	11.3	11.5	12.0	11.7	11.2	11.3	11.8	11.4	12.1	11.4	12.0
14	15.8	17.5	15.1	17.4	14.8	17.4	17.3	14.2	17.1	17.3	15.7	17.2	14.1	17.2	14.8	17.6	15.0	17.5
15	25.4	28.5	22.7	25.3	25.4	26.5	28.5	22.7	24.1	25.3	25.3	28.4	22.7	25.2	25.3	28.4	22.7	25.3
CH_3COO	170.5	154.5	170.4	154.7							170.6	154.5	171.3	154.7	171.3	154.2	170.5	154.1
CH_3COO	21.1	19.5	21.0	19.5							21.1	19.5	21.1	19.6	21.1	19.7	21.1	19.7
CH_3COO	171.2	154.1	171.3	154.1														
CH_3COO	21.1	19.8	21.1	19.8														

[a] Values calculated at the B3LYP/6-31G*//B3LYP/6-31G* theoretical level.

we evaluated systematic MM+ geometries and B3LYP/6-31G* electronic properties (B3LYP/6-31G*//MM+) for the larger acetylated sulfite derivatives. The theoretical geometries are presented in Table 2, including the puckering parameters[31] of the different rings. Special emphasis has placed on the sulfite C-ring. The geometrical features for this ring remained almost unchanged upon acetylation, but yielding noticeable and systematic variations according to the *R*/*S* sulfur configuration. The sulfinyl S−O distance was shorter (ca. 0.15 Å) for the *R* configuration.

In terms of puckering parameters, A- and B-rings had almost undistorted $^4C_1$ conformations with large puckering amplitude $Q \approx 0.56$ Å and very small Θ values (<0.7 °). The C-ring presented also a $^4C_1$ distorted conformation for the *R* isomer; however, the *S*-isomer (equatorial oxygen) had the same $^4C_1$ but with a distorted ring conformation (higher ring puckering amplitude $Q > 0.68$ Å and $\Theta \approx 13$ °). The main geometrical differences between MM+ and the B3LYP/6-31G* methods appeared in the sulfite bond description, due to the lack of parametrization of the MM+ for different S−O arrangements.

To validate the B3LYP/6-31G*//MM+ scheme for the sulfites derivatives, we compared the theoretical and experimental δ_H and δ_C shifts for **8**, **9**, **12**−**15**, **17**, and **18**. The numerical data are presented in Tables 4 and 5 for these shifts, including the experimental data for comparison, and also the B3LYP/6-31G*//B3LYP/6-31G* values for **12** and **15**. The B3LYP/6-31G*//B3LYP/6-31G* and B3LYP/6-31G*//MM+ theoretical δ_C shifts for **12** and **15** matched very well, the main differences appearing in the C-4 and C-6 atoms bonded to the sulfite group. However, the trends for these shifts were the same as in the experimental data. The excellent agreement between the experimental and theoretical shifts for **12** and **15** at the B3LYP/6-31G*//B3LYP/6-31G* is remarkable, with a mean deviation <1.6 ppm compared with <4.4 ppm for the B3LYP/6-31G*//MM+ results, indicating the necessity to validate the B3LYP/6-31G*//MM+ results with B3LYP/6-31G*//B3LYP/6-31G* calculations when nonstandard functional groups are present. The theoretical and experimental δ_C shifts for all the compounds were in very good agreement, allowing us the unequivocal *R*/*S* configurational assignment for these structures. The main differences between the *R* and *S* isomers were in the C-6 and C-5 shifts. The *S*-sulfite had the C-6 deshielded by 8 ppm experimentally (7 ppm theoretically) with respect to the *R*-sulfite. However, the C-15 signal had opposite trends with deshielded values (ca. 3 ppm) for the *R*-sulfite (both theoretically and experimentally).

The theoretical δ_H shifts together with their corresponding coupling constants, listed in Table 5, show excellent agreement with the available experimental data, regardless of the theoretical method employed. These results confirmed the overall stereochemistry of the structures studied. In addition, the *R*/*S* configuration (e.g., 4.8(5.1) and 4.4(4.1) ppm for δ_6 of **8** and **9**, respectively; theoretical values in parentheses). Moreover, several theoretical data have no corresponding experimental values available, pointing out the utility of these data for future assignments when better resolved spectra become available).

Giving that the $^3J_{HH}$ coupling constants are highly sensitive to the geometries, these constants are an excellent test for the quality of the theoretical geometries reported. Thus, for the structures studied a very good agreement between the experimental and theoretical data was found, supporting the goodness of the B3LYP/6-31G*//MM+ and B3LYP/6-31G*//B3LYP/6-31G* results (see Table 5).

III. Conclusions

Taking into account the enzymatic essays performed on the polyhydroxyeudesmane from a natural source, several enzymatic methods have been developed for regioselective protection on C-1 and C-12 or C-12 centers. From these products, suitably protected, two stereoisomers sulfites were obtained. Hence, observable spectroscopic data are confirmed with values obtained using theoretical methods. Moreover, the two sulfites reacted

(30) A searching in the Cambridge Structural Database gave only one structure with a six-membered sulfite triterpene derivative; see Duax, W. L.; Griffin, J. F.; Wolff, M. E. *Cryst. Struct. Commun.* **1976**, *5*, 279.

(31) Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354

Table 5. Experimental and Calculated (B3LYP/6-31G*//MM+) NMR ^{1}H Chemical Shifts (ppm) Together with Their Coupling Constants (Hz)

	8 *R* (axial)		**9** *S* (equatorial)		**12** *R* (axial)			**15** *S* equatorial)			**17** *R* (axial)		**14** *S* (equatorial)		**18** *R* (axial)		**13** *S* (equatorial)	
δ_C	exptl	calcd	exptl	calcd	exptl	calcd[a]	calcd	exptl	calcd[a]	calcd	exptl	calcd	exptl	calcd	exptl	calcd	exptl	calcd
δ_1	4.6	4.1	4.6	4.2	3.4	3.5	3.3	3.4	3.5	3.4	4.6	4.1	3.4	4.2	3.4	3.3	4.6	3.3
$J_{12\alpha}$	4.3	5.1	4.2	5.0	4.5	4.4	5.5	3.4	4.3	5.4	4.6	5.1	4.3	5.0	4.4	5.5	4.2	5.4
$J_{12\beta}$	10.9	10.8	11.3	10.9	10.9	11.1	10.5	10.6	11.2	10.6	10.3	10.8	11.2	10.9	10.8	10.5	12.7	10.6
$\delta_{2\alpha}$		1.9		2.0		1.7	1.7		1.8	1.8		2.0		2.0		1.7		1.8
$\delta_{2\beta}$		1.5		1.4		1.7	1.7		1.7	1.7		1.5		1.4		1.7		1.7
$J_{2\alpha3\alpha}$		4.3		4.1		3.9	4.4		4.0	4.2		4.3		4.1		4.4		4.2
$J_{2\alpha3\beta}$		2.7		2.8		2.6	2.7		2.6	2.8		2.8		2.8		2.7		2.7
$J_{2\beta3\alpha}$		13.4		13.5		13.7	13.4		13.7	13.5		13.4		13.5		13.4		13.5
$J_{2\beta3\beta}$		4.3		4.2		4.0	4.1		4.3	4.2		4.2		4.2		4.3		4.2
$\delta_{3\alpha}$		1.6		1.7		1.6	1.5		1.7	1.6		1.6		1.7		1.5		1.6
$\delta_{3\beta}$		1.6		1.6		1.7	1.6		1.7	1.7		1.6		1.6		1.6		1.7
δ_5	2.0	1.6	2.3	1.8	1.9	2.0	1.5	2.2	1.8	1.7	2.0	1.7	2.2	1.8	1.9	1.5	2.3	1.6
J_{56}	10.3	11.1	10.8	11.3	10.3	10.9	11.1	10.7	11.1	11.3	10.3	11.1	10.8	11.3	10.3	11.1	10.7	11.3
δ_6	4.8	5.1	4.4	4.1	4.9	5.2	5.1	4.5	4.5	4.1	4.9	5.1	4.4	4.1	4.9	5.1	4.5	4.1
J_{67}	10.3	10.2	10.8	10.3	10.3	9.9	10.2	10.7	10.1	10.3	10.3	10.2	10.8	10.3	10.3	10.1	10.7	10.3
δ_7		2.1		2.0		2.5	2.3		2.5	2.3		2.4		2.4		2.0		1.9
$J_{78\alpha}$		3.2		3.1		3.4	3.4		3.2	3.1		3.2		3.1		3.2		3.1
$J_{78\beta}$		12.3		12.4		12.3	12.3		12.3	12.4		12.4		12.4		12.3		12.4
$\delta_{8\alpha}$		1.4		1.4		1.7	1.6		1.7	1.6		1.5		1.5		1.4		1.4
$\delta_{8\beta}$		1.5		1.3		1.5	1.5		1.4	1.4		1.5		1.3		1.5		1.4
$J_{8\alpha9\alpha}$		3.3		3.3		3.4	3.2		3.6	3.3		3.2		3.3		3.3		3.3
$J_{8\alpha9\beta}$		3.6		3.6		3.3	3.7		3.1	3.7		3.7		3.7		3.6		3.7
$J_{8\beta9\alpha}$		13.4		13.3		13.4	13.4		13.3	13.4		13.4		13.4		13.4		13.4
$J_{8\beta9\beta}$		3.0		3.0		3.3	3.0		3.5	3.0		3.0		3.0		3.0		3.0
$\delta_{9\alpha}$		1.1		1.1		1.2	1.1		1.2	1.1		1.2		1.2		1.0		1.0
$\delta_{9\beta}$		1.8		1.8		1.6	1.6		1.5	1.6		1.8		1.8		1.6		1.6
δ_{15}	1.8	1.9	1.6	1.5		1.9	1.9		1.5	1.5		1.9		1.5		1.9		1.5

[a] Values calculated at the B3LYP/6-31G*//B3LYP/6-31G* theoretical level.

differently by incubation with *R. nigricans* and by redox reactions. Furthermore, the R-sulfite biotransformation yielded metabolites with hydroxy groups at 2α and 11*β*. These functionalizations enable us to produce derivatives of selinanetetrols.

The accurate theoretical calculations performed on the *R*/*S* sulfite pair (**12** and **15**) yielded geometries with the three rings in an almost standard $^4C_1$ conformation. The theoretical δ_H and δ_C shifts were compared with the experimental data, allowing us the correct assignation of the *R*/*S* configurational sulfite derivatives. The B3LYP/6-31G*//B3LYP/6-31G* theoretical level was used together with the easily available B3LYP/6-31G*//MM+ method for **12** and **15**, with very good agreement. The results were also extended for the acetylated derivatives. The main differences (theoretical vs experimental) appeared for the C-4 and C-6 δ_C shifts. However, the ^{1}H spectra were remarkably well described in both δ_H and $^3J_{HH}$ coupling constants.

Experimental Section

Computational Details. All the structures were fully optimized with the MM+ force field[25] using the Hyperchem program.[32] The ^{13}C and ^{1}H NMR chemical shifts were calculated with the GIAO method,[26] using their corresponding TMS shielding as reference. Density functional B3LYP/6-31G* single-point calculations were performed on the optimized MM+ geometries (denoted as B3LYP/6-31G*//MM+) with the Gaussian 98 package of programs[33] in order to reproduce the δ_C and δ_H chemical shifts. In addition, the calculations at the B3LYP/6-31G*//B3LYP/6-31G* level were performed on **12** and **15**, followed by calculations of the δ_C and δ_H shifts at the same levels. The $^3J_{HH}$ coupling constants have been calculated using the equation of Haasnoot-Leeuw-Altona.[29]

General Experimental Procedures. 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 an AM-300 spectrometer. 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°. One-dimensional NOE difference experiments were made by irradiation for 4 s in series of 8 scans. Mass spectra were determined with CI (methane). Mps are uncorrected. Optical rotations were measured on a polarimeter at 25 °C. Silica gel 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) were rendered visible by spraying with H_2SO_4−AcOH, followed by heating to 120 °C. Lipase (type II, crude) from pig pancreas (PPL) (190 units/mg protein), CCL (powder, Lipase type VII from *C. cylindracea*, 700−1500 units/mg protein) were purchased from Aldrich Chemical Co.). Lipozyme (MML) (lipase IM-60 from *Mucor miehei* in the immobilized form on a microporous anion-

(32) Hyperchem(TM) release 4.5 for SGI, 1995 Hypercube, Inc.

(33) Gaussian 98, revision A.6 Frisch,. M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1998.

exchange resin) and *C. antarctica* lipase (Novozym 435 acrylic resin supported lipase produced by host organism *Aspergillus oryzae*, after transfer of the genetic coding for lipase B from *C. antarctica*) were generous gifts of Novo Nordisk Bioindustrial Group. Lipase A (powder, lipase from *Aspergillus niger*, 60,000 u/g); lipase AYS (powder, lipase from *Candida rugosa*, 30,000 u/g); Newlase F (powder, lipase from *Rhizopus niveus*), 30.000 u/g); lipase AK (powder, lipase from *Pseudomonas fluorescens*, 20,000 u/g); lipase PS (powder, lipase from *Pseudomonas cepacia*, 30,000 u/g) were generously given by Amano Enzyme LTD Co.

Isolation and Reduction of Vulgarin (1). Vulgarin (**1**)[28] was isolated from *Artemisia canariensis* and it was hydrogenated with hydrogen on PtO_2 to obtain 1*β*,4α-dihydroxy-5α,11*β*-*H*-eudesman-6α,12-olide (**2**).[18] Reduction of **2** with $LiAlH_4$/THF gave 1*β*,4α,6α,12-tetrahydroxy-5α,11*β*-*H*-eudesmane (**3**)[18] which was acetylated with Ac_2O/Py at room temperature to give 1*β*,12-diacetoxy-4α,6α-dihydroxy-5α,11*β*-*H*-eudesmane (**4**)[18] and 1*β*,6α,12-triacetoxy-4α-hydroxy-5α,11*β*-*H*-eudesmane (**5**).[18]

Tests of Enzymatic Acetylation of 1*β*,4α,6α,12-tetrahydroxy-5α,11*β*-*H*-eudesmane (2) with Lipases. Nine samples of 100 mg each of **3** were dissolved in 7 mL each of vinyl acetate, and then 600 mg of nine lipases indicated in Table 1 were added. The different suspensions were shaken on an orbital shaker at 45 °C for 1, 8, 24, 48, 96 and 168 h controlling the proportion of transformation by ^{1}H and ^{13}C NMR. The results of these tests are shown in Table 1. When the enzymatic reaction was stopped, the reaction mixture was filtered and the different products were isolated by flash chromatography. Thus, mono-, di- and triacetylated compounds **4**,[18] **5**,[18] **6** and **7** were obtained from these enzymatic reactions. 12-Acetoxy-1*β*,4α,6α-trihydroxy-5α,11*β*-*H*-eudesmane (**6**): white solid; mp 111−113 °C; $[\alpha]^{25}_D = -16$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 3347, 1737, 1243, 1067 cm^{-1}; ^{1}H NMR ($CDCl_3$) *δ* 3.96 (1H, dd, $J_1 = 7.3$ Hz, $J_2 = 10.8$ Hz, H-12), 3.89 (1H, dd, $J_1 = 7.1$ Hz, $J_2 = 10.8$ Hz, H-12), 3.85 (1H, dd, $J_1 = J_2 = 10.3$ Hz, H-6*β*), 3.27 (1H, dd, $J_1 = 4.3$ Hz, $J_2 = 10.6$ Hz, H-1α), 2.37 (1H, dq, $J_1 = 7.0$ Hz, $J_2 = 14.2$ Hz, H-11*β*), 2.02 (3H, s, *Me*CO), 1.30 (3H, s, 3H-15), 0.86 (3H, d, $J = 7.0$ Hz, 3H-13), 0.81 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) *δ* 11.1 (C-13), 14.0 (C-14), 19.1 (C-8), 21.1 (*Me*CO), 23.5 (C-15), 28.6 (C-2), 30.4 (C-11), 39.1 (C-3), 40.3 (C-10), 40.4 (C-9), 46.8 (C-7), 56.0 (C-5), 66.5 (C-12), 70.7 (C-6), 73.5 (C-4), 80.4 (C-1), 170.9 (Me*C*O); HRMS calcd for $C_{17}H_{30}O_5Na$ 337.1991, found 337.1996. 1*β*-Acetoxy-4α,6α,12-trihydroxy-5α,11*β*-*H*-eudesmane (**7**): white solid; mp 158−160 °C; $[\alpha]^{25}_D = -26$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 3312, 1735, 1246, 1056 cm^{-1}; ^{1}H NMR ($CDCl_3$) *δ* 4.54 (1H, dd, $J_1 = 4.1$ Hz, $J_2 = 11.1$ Hz, H-1α), 3.90 (1H, dd, $J_1 = J_2 = 10.2$ Hz, H-6*β*), 3.55 (1H, dd, $J_1 = 5.6$ Hz, $J_2 = 10.7$ Hz, H-12), 3.47 (1H, dd, $J_1 = 7.9$ Hz, $J_2 = 10.7$ Hz, H-12), 2.10 (1H, dq, $J_1 = 6.9$ Hz, $J_2 = 14.3$ Hz, H-11*β*), 2.02 (3H, s, *Me*CO), 1.35 (3H, s, 3H-15), 0.92 (3H, s, 3H-14), 0.88 (3H, d, $J = 6.9$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$) *δ* 12.6 (C-13), 15.0 (C-14), 20.5 (C-8), 21.3 (*Me*CO), 23.5 (C-15), 24.8 (C-2), 35.5 (C-11), 39.1 (C-3), 39.3 (C-10), 39.9 (C-9), 46.8 (C-7), 56.3 (C-5), 66.5 (C-12), 70.7 (C-6), 73.5 (C-4), 80.4 (C-1), 170.9 (Me*C*O); HRMS calcd for $C_{17}H_{30}O_5Na$ 337.1991, found 337.1993.

Formation of Sulfites 8 and 9. Product **4** (600 mg) was dissolved in 7 mL of pyridine, and 0.61 mL of Cl_2SO was added. The reaction was maintained with stirring at 0 °C for 30 min. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$, neutralized with saturated aqueous $NaHCO_3$, dried with anhydrous Na_2SO_4 , and evaporated at reduced pressure. Chromatography over silica gel yielded 1*β*,12-diacetoxy-5α,11*β*-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**8**) (237 mg, 35%): colorless oil; $[\alpha]^{25}_D = -44$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 1737, 1034, 1003 cm^{-1}; ^{1}H NMR ($CDCl_3$) *δ* 4.84 (1H, dd, $J_1 = J_2 = 10.3$ Hz, H-6*β*), 4.59 (1H, dd, $J_1 = 4.3$ Hz, $J_2 = 10.9$ Hz, H-1α), 3.98 (1H, dd, $J_1 = 7.0$ Hz, $J_2 = 10.8$ Hz, H-12), 3.94 (1H, dd, $J_1 = 7.7$ Hz, $J_2 = 10.8$ Hz, H-12), 2.28 (1H, dq, $J_1 = 7.1$ Hz, $J_2 = 14.3$ Hz, H-11*β*), 2.04, 2.03 (1H, d, $J = 10.3$ Hz, H-5α), 2.02 (3H each, s, *Me*CO), 1.81 (3H, s, 3H-15), 0.95 (3H, s, 3H-14), 0.90 (3H, d, $J = 7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$) *δ* 11.4 (C-13), 15.8 (C-14), 18.1 (C-8), 21.0 (*Me*CO), 21.1 (*Me*CO), 23.9 (C-2), 25.4 (C-15), 30.5 (C-11), 38.3 (C-10), 38.6 (C-3), 39.3 (C-9), 43.1 (C-7), 52.9 (C-5), 66.1 (C-6), 67.2 (C-12), 79.3 (C-1), 81.6 (C-4), 170.5 (Me*C*O), 171.2 (Me*C*O); HRMS calcd for $C_{19}H_{30}O_7NaS$ 425.1610, found 425.1611. 1*β*,12-Diacetoxy-5α,-11*β*-*H*-eudesman-4α,6α-diyl-S(*S*)-cyclic sulfite (**9**) (305 mg, 45%): white solid; mp 104−106 °C; $[\alpha]^{25}_D = -51$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1735, 1034, 1001 cm^{-1}; ^{1}H NMR (*δ*, $CDCl_3$) 4.61 (1H, dd, $J_1 = 4.2$ Hz, $J_2 = 11.3$ Hz, H-1α), 4.43 (1H, dd, $J_1 = J_2 = 10.8$ Hz, H-6*β*), 3.98 (1H, dd, $J_1 = 6.9$ Hz, $J_2 = 10.9$ Hz, H-12), 3.92 (1H, dd, $J_1 = 7.8$ Hz, $J_2 = 10.9$ Hz, H-12), 2.42 (1H, dq, $J_1 = 7.1$ Hz, $J_2 = 14.5$ Hz, H-11*β*), 2.32 (1H, d, $J = 10.8$ Hz, H-5α), 2.04, 2.03 (3H each, s, *Me*CO), 1.65 (3H, s, 3H-15), 0.93 (3H, s, 3H-14), 0.90 (3H, d, $J = 7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$) *δ* 11.3 (C-13), 15.1 (C-14), 18.9 (C-8), 21.0 (*Me*CO), 21.1 (*Me*CO), 22.7 (C-15), 24.3 (C-2), 30.3 (C-11), 38.4 (C-9), 38.9 (C-10), 39.1 (C-3), 44.6 (C-7), 50.3 (C-5), 67.1 (C-12), 74.7 (C-6), 79.2 (C-1), 82.6 (C-4), 170.4 (Me*C*O), 171.3 (Me*C*O); HRMS calcd for $C_{19}H_{30}O_7NaS$ 425.1610, found 425.1607.

Organism, Media, and Culture Conditions. *R. nigricans* 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 5.0. 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 °C for 6 days, after which substrates **8** and **9** in EtOH were added.

Biotransformation of Substrate 8 with *R. nigricans*. Substrate **8** (220 mg) was dissolved in EtOH (6 mL), distributed among 6 Erlenmeyer-flask cultures and incubated for 14 days, after which time 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 °C in a vacuum to give a mixture of compounds. This mixture was chromatographed on a silica gel column to obtain 35 mg of 1*β*,12-diacetoxy-2α-hydroxy-5α,11*β*-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**10**, 15%): colorless oil; $[\alpha]^{25}_D = -31$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1738, 1238, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$) *δ* 4.87 (1H, dd, $J_1 = J_2 = 10.3$ Hz, H-6*β*), 4.61 (1H, d, $J = 9.7$ Hz, H-1α), 3.96 (1H, dd, $J_1 = 7.0$ Hz, $J_2 = 10.9$ Hz, H-12), 3.92 (1H, dd, $J_1 = 7.8$ Hz, $J_2 = 10.9$ Hz, H-12), 3.78 (1H, ddd, $J_1 = 4.9$ Hz, $J_2 = 9.7$ Hz; $J_3 = 11.8$ Hz, H-2*β*), 2.12, 2.10 (1H, d, $J = 10.3$ Hz, H-5α), 2.05 (3H each, s, *Me*CO), 1.84 (3H, s, 3H-15), 0.96 (3H, s, 3H-14), 0.90 (3H, d, $J = 7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$) *δ* 11.4 (C-13), 16.7 (C-14), 18.2 (C-8), 21.0 (*Me*CO), 21.1 (*Me*CO), 26.7 (C-15), 30.5 (C-11), 38.5 (C-10), 39.4 (C-9), 43.0 (C-7), 47.7 (C-3), 52.9 (C-5), 66.4 (C-6), 67.1 (C-12), 67.1 (C-2), 80.6 (C-4), 83.9 (C-1), 171.2 (Me*C*O), 171.8 (Me*C*O); HRMS calcd for $C_{19}H_{30}O_8NaS$ 441.1559, found 441.1558. Also obtained was 42 mg of 1*β*-acetoxy-11*β*,12-dihydroxy-5α-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**11**, 20%): colorless oil; $[\alpha]^{25}_D = -35$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) *δ* 5.29 (1H, dd, $J_1 = 9.4$ Hz, $J_2 = 11.0$ Hz, H-6*β*), 4.58 (1H, dd, $J_1 = 4.6$ Hz, $J_2 = 11.0$ Hz, H-1α), 3.52 (1H, d, $J = 11.3$ Hz, H-12), 3.40 (1H, d, $J = 11.3$ Hz, H-12), 2.13 (1H, d, $J = 11.0$ Hz, H-5α), 2.03 (3H, s, *Me*CO), 1.85 (3H, s, 3H-15), 1.20 (3H, s, 3H-13), 0.96 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) *δ* 15.7 (C-14), 20.4 (C-8), 21.1 (*Me*CO), 21.4 (C-13), 23.9 (C-2), 25.3 (C-15), 38.1 (C-10), 38.6 (C-3), 39.1 (C-9), 47.0 (C-7), 52.7 (C-5), 67.7 (C-6), 68.8 (C-12), 74.7 (C-11), 79.1 (C-1), 82.6 (C-4), 170.5 (Me*C*O); HRMS calcd for $C_{17}H_{28}O_7NaS$ 399.1453, found 399.1456. Also obtained was 87 mg of 1*β*,12-dihydroxy-5α,-11*β*-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**12**, 50%): white solid; mp 174−176 °C; $[\alpha]^{25}_D = -54$ (*c* 1, MeOH); IR ($CHCl_3$) 3400, 1242, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$) *δ* 4.88 (1H, dd, $J_1 = J_2 = 10.3$ Hz, H-6*β*), 3.53 (1H, dd, $J_1 = 6.2$ Hz, $J_2 = 10.7$ Hz, H-12), 3.47 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 10.7$ Hz, H-12), 3.37 (1H, dd, $J_1 = 4.5$ Hz, $J_2 = 10.9$ Hz, H-1α), 2.07 (1H, d, $J_1 = 6.9$ Hz, $J_2 = 14.3$ Hz, H-11*β*), 1.90 (1H, d, $J = 11.3$ Hz, H-5α),

1.79 (3H, s, 3H-15), 0.88 (3H, s, 3H-14), 0.87 (3H, d, J = 6.9 Hz, 3H-13); ^{13}C NMR ($CDCl_3$) δ 11.7 (C-13), 14.8 (C-14), 18.7 (C-8), 25.4 (C-15), 27.4 (C-2), 34.4 (C-11), 38.9 (C-3), 39.3 (C-10), 39.5 (C-9), 42.6 (C-7), 52.9 (C-5), 66.0 (C-12), 67.1 (C-6), 78.4 (C-1), 82.2 (C-4); HRMS calcd for $C_{15}H_{26}O_5NaS$ 341.1399, found 341.1392. Also obtained was 8 mg of 1β,4α,6α,12-tetrahydroxy-5α,11β-*H*-eudesmane (**3**, 5%).

Biotransformation of Substrate 9 with *R. nigricans*. Substrate **9** (260 mg) was dissolved in EtOH (7 mL), distributed among seven Erlenmeyer-flask cultures, and incubated for 14 days, after which time 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 °C in a vacuum to give a mixture of compounds. This mixture was chromatographed on a silica gel column to obtain 35 mg of 1β-acetoxy-12-hydroxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(***S***)-cyclic sulfite (**13**, 15%): colorless oil; $[\alpha]^{25}_D = -55$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 3476, 1733, 1240, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.59 (1H, dd, J_1 = 4.2 Hz, J_2 = 12.7 Hz, H-1α), 4.46 (1H, dd, $J_1 = J_2$ = 10.7 Hz, H-6β), 3.55 (1H, dd, J_1 = 6.7 Hz, J_2 = 10.7 Hz, H-12), 3.50 (1H, dd, J_1 = 7.3 Hz, J_2 = 10.7 Hz, H-12), 2.28 (1H, d, J = 11.7 Hz, H-5α), 2.20 (1H, dq, J_1 = 7.0 Hz, J_2 = 14.3 Hz, H-11β), 2.03 (3H, s, *Me*CO), 1.66 (3H, s, 3H-15), 0.93 (3H, d, J = 7.0 Hz, 3H-13), 0.89 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ 11.4 (C-13), 15.1 (C-14), 19.2 (C-8), 21.1 (*Me*CO), 22.7 (C-15), 24.3 (C-2), 34.0 (C-11), 38.3 (C-3), 38.9 (C-10), 39.2 (C-9), 44.4 (C-7), 50.5 (C-5), 66.3 (C-12), 75.2 (C-6), 79.3 (C-1), 82.8 (C-4), 170.5 (Me*C*O); HRMS calcd for $C_{17}H_{28}O_6NaS$ 383.1504, found 383.1503. Also obtained was 24 mg of 12-acetoxy-1β-hydroxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(***S***)-cyclic sulfite (**14**, 10%): colorless oil; $[\alpha]^{25}_D$ = −136 (c 0.5, $CHCl_3$); ($CDCl_3$); IR ($CHCl_3$) 3504, 1735, 1236, 1035 cm^{-1}; ^{1}H NMR δ 4.44 (1H, dd, $J_1 = J_2$ = 10.8 Hz, H-6β), 3.98 (1H, dd, J_1 = 7.0 Hz, J_2 = 10.9 Hz, H-12), 3.93 (1H, dd, J_1 = 7.7 Hz, J_2 = 10.9 Hz, H-12), 3.40 (1H, dd, J_1 = 4.3 Hz, J_2 = 11.2 Hz, H-1α), 2.42 (1H, dq, J_1 = 7.1 Hz, J_2 = 14.3 Hz, H-11β), 2.24 (1H, d, J = 10.8 Hz, H-5α), 2.05 (3H, s, *Me*CO), 1.62 (3H, s, 3H-15), 0.91 (3H, d, J = 7.1 Hz, 3H-13), 0.86 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ 11.3 (C-13), 14.1 (C-14), 19.2 (C-8), 21.1 (*Me*CO), 22.7 (C-15), 27.8 (C-2), 30.3 (C-11), 38.7 (C-3), 39.3 (C-9), 39.9 (C-10), 44.9 (C-7), 50.2 (C-5), 67.3 (C-12), 75.1 (C-6), 78.3 (C-1), 83.1 (C-4), 171.3 (Me*C*O); HRMS calcd for $C_{17}H_{28}O_6NaS$ 383.1504, found 383.1509. Also obtained was 123 mg of 1β,12-dihydroxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*S*)-cyclic sulfite (**15**, 60%): white solid; mp 195−197 °C dec; $[\alpha]^{25}_D = -82$ (c 0.5, MeOH); IR ($CHCl_3$) 3397, 1265, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.46 (1H, dd, $J_1 = J_2$ = 10.7 Hz, H-6β), 3.54 (1H, dd, J_1 = 6.6 Hz, J_2 = 10.8 Hz, H-12), 3.47 (1H, dd, J_1 = 8.1 Hz, J_2 = 10.8 Hz, H-12), 3.40 (1H, dd, J_1 = 3.4 Hz, J_2 = 10.6 Hz, H-1α), 2.18 (1H, dq, J_1 = 7.0 Hz, J_2 = 14.3 Hz, H-11β), 2.18 (1H, d, J = 10.7 Hz, H-5α), 1.65 (3H, s, 3H-15), 0.87 (3H, d, J = 7.0 Hz, 3H-13), 0.85 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ 11.3 (C-13), 14.2 (C-14), 19.1 (C-8), 22.7 (C-15), 27.7 (C-2), 33.8 (C-11), 38.7 (C-3), 39.4 (C-9), 39.9 (C-10), 44.3 (C-7), 50.5 (C-5), 66.1 (C-12), 75.6 (C-6), 78.3 (C-1), 83.4 (C-4); HRMS calcd for $C_{15}H_{26}O_5NaS$ 341.1399, found 341.1397. Also obtained was 4 mg of 6α-sulfinyloxy-1β,12-dihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**16**, 5%): colorless oil; $[\alpha]^{25}_D = -30$ (c 0.5, MeOH); IR ($CHCl_3$) 3430, 1032, 1001 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.03 (1H, bs, H-15), 4.73 (1H, bs, H-15), 3.76 (1H, dd, $J_1 = J_2$ = 10.7 Hz, H-6β), 3.64 (1H, dd, J_1 = 5.5 Hz, J_2 = 10.8 Hz, H-12), 3.50 (1H, dd, J_1 = 7.1 Hz, J_2 = 10.8 Hz, H-12), 3.42 (1H, dd, J_1 = 4.7 Hz, J_2 = 11.5 Hz, H-1α), 0.92 (3H, d, J = 7.0 Hz, 3H-13), 0.70 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ 10.6 (C-13), 12.3 (C-14), 20.0 (C-8), 29.8 (C-2), 32.9 (C-3), 34.3 (C-9), 35.6 (C-11), 39.6 (C-10), 47.2 (C-7), 53.1 (C-5), 66.8 (C-12), 76.1 (C-6), 76.6 (C-1), 108.6 (C-4), 144.0 (C-15); HRMS calcd for $C_{15}H_{26}O_5NaS$ 341.1397, found 341.1398. Also obtained was 9 mg of 1β,4α,6α,12-tetrahydroxy-5α,11β-*H*-eudesmane (**3**, 5%).

Treatment of 1β,12-Diacetoxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic Sulfite (8) with $NaBH_4$. Product **8** (100 mg) was dissolved in 5 mL of EtOH, and 20 mg of $NaBH_4$ was added. The mixture reaction was maintained with stirring at room temperature for 12 h. The reaction mixture was washed with diluted HCl solution, extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4, and evaporated at reduced pressure. Chromatography over silica gel yielded 29 mg of 1β-acetoxy-12-hydroxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**17**, 32%): white solid; mp 156−158 °C; $[\alpha]^{25}_D = -57$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 3475, 1730, 1240, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.87 (1H, dd, $J_1 = J_2$ = 10.3 Hz, H-6β), 4.58 (1H, dd, J_1 = 4.6 Hz, J_2 = 11.0 Hz, H-1α), 3.54 (1H, dd, J_1 = 6.3 Hz, J_2 = 10.7 Hz, H-12), 3.47 (1H, dd, J_1 = 8.0 Hz, J_2 = 10.7 Hz, H-12), 2.08 (1H, dq, J_1 = 7.0 Hz, J_2 = 14.4 Hz, H-11β), 2.03 (1H, d, J = 10.3 Hz, H-5α), 2.02 (3H, s, *Me*CO), 1.81 (3H, s, 3H-15), 0.95 (3H, s, 3H-14), 0.87 (3H, d, J = 7.0 Hz, 3H-13); ^{13}C NMR ($CDCl_3$) δ 11.7 (C-13), 15.8 (C-14), 18.6 (C-8), 21.1 (*Me*CO), 23.9 (C-2), 25.3 (C-15), 34.4 (C-11), 38.3 (C-10), 38.6 (C-3), 39.3 (C-9), 42.5 (C-7), 53.0 (C-5), 66.7 (C-12), 66.7 (C-6), 79.4 (C-1), 81.7 (C-4), 170.6 (Me*C*O); HRMS calcd for $C_{17}H_{28}O_6NaS$ 383.1504, found 383.1496. Also found was 13 mg of 12-acetoxy-1β-hydroxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic sulfite (**18**, 15%): syrup; $[\alpha]^{25}_D = -26$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 3503, 1735, 1234, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.86 (1H, dd, $J_1 = J_2$ = 10.3 Hz, H-6β), 3.97 (1H, AB collapsed, 2H-12), 3.94 (1H, AB collapsed, H-12), 3.38 (1H, dd, J_1 = 4.4 Hz, J_2 = 10.8 Hz, H-1α), 2.29 (1H, dq, J_1 = 7.0 Hz, J_2 = 14.3 Hz, H-11β), 2.05 (3H, s, *Me*CO), 1.90 (1H, d, J = 10.3 Hz, H-5α), 1.79 (3H, s, 3H-15), 0.91 (3H, d, J = 7.0 Hz, 3H-13), 0.89 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$) δ 11.4 (C-13), 14.8 (C-14), 18.4 (C-8), 21.1 (*Me*CO), 25.4 (C-15), 27.4 (C-2), 30.6 (C-11), 38.9 (C-3), 39.3 (C-10), 39.5 (C-9), 43.3 (C-7), 52.8 (C-5), 66.5 (C-6), 67.4 (C-12), 78.4 (C-1), 82.0 (C-4), 171.3 (Me*C*O); HRMS calcd for $C_{17}H_{28}O_6NaS$ 383.1504, found 383.1500. Also found was 3 mg of **12** (3%).

Treatment of 1β,12-Diacetoxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic Sulfite (9) with $NaBH_4$. Product **9** (100 mg) was dissolved in 5 mL of EtOH and 20 mg of $NaBH_4$ were added. The mixture reaction was maintained with stirring at room temperature for 12 h. The reaction mixture was washed with diluted HCl solution, extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4 , and evaporated at reduced pressure. Chromatography over silica gel yielded 18 mg of **13** (20%), 7 mg of **14** (8%), and 2 mg of **15** (2%).

Oxidation with $NaIO_4$/$RuCl_3$ of 1β,12-Acetoxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*R*)-cyclic Sulfite (8). $NaIO_4$ (32 mg) and $RuCl_3 \cdot 3H_2O$ (approximately 1 mg) in water (1 mL) were added to a solution of **8** (40 mg) in CCl_4 (0.5 mL) and CH_3CN (0.5 mL). The reaction mixture was stirred at 25 °C for 12 h. This mixture was diluted with ethyl ether (4 mL), washed with water, and the organic layer was washed with water, a saturated solution of $NaHCO_3$, and a NaCl solution, and dried over anhydrous Na_2SO_4. The solvent was evaporated at reduced pressure, and the residue was chromatographed to obtain 42 mg (**19**, 100%) of 1β,12-diacetoxy-5α,11β-*H*-eudesman-4α,6α-diyl-S-cyclic sulfate: syrup; $[\alpha]^{25}_D = -40$ (*c* 1, $CHCl_3$); IR ($CHCl_3$) 1735, 1230, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.77 (1H, dd, $J_1 = J_2$ = 10.5 Hz, H-6β), 4.60 (1H, dd, J_1 = 4.5 Hz, J_2 = 11.5 Hz, H-1α), 3.97 (1H, dd, J_1 = 6.5 Hz, J_2 = 11.0 Hz, H-12), 3.92 (1H, dd, J_1 = 7.8 Hz, J_2 = 11.0 Hz, H-12), 2.34 (1H, dq, J_1 = 7.1 Hz, J_2 = 14.7 Hz, H-11β), 2.04 and 2.03 (3H each, s, *Me*CO), 1.98 (1H, d, J = 10.5 Hz, H-5α), 1.77 (3H, s, 3H-15), 0.93 (3H, s, 3H-14), 0.91 (3H, d, J = 7.1 Hz, 3H-13); ^{13}C NMR ($CDCl_3$) δ 11.3 (C-13), 15.6 (C-14), 18.5 (C-8), 21.0 (*Me*CO), 21.0 (*Me*CO), 22.0 (C-15), 24.7 (C-2), 30.4 (C-11), 37.8 (C-3), 38.9 (C-9), 39.2 (C-10), 43.1 (C-7), 51.2 (C-5), 66.6 (C-12), 78.7 (C-1), 81.2 (C-6), 91.7 (C-4), 170.3 (Me*C*O), 171.1 (Me*C*O); HRMS calcd for $C_{19}H_{30}O_8NaS$, 441.1559, found 441.1565.

Oxidation with $NaIO_4$/$RuCl_3$ of 1β,12-Acetoxy-5α,11β-*H*-eudesman-4α,6α-diyl-S(*S*)-cyclic Sulfite (9). A similar oxidative process was carried out on **9** (50 mg) but now 3 h were required to complete the reaction and so 52 mg (100%) of **19** was obtained.

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Supporting Information Available: ^{13}C spectra for all new compounds **6**−**19**. This material is available free of charge via Internet at http://pubs.acs.org.

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