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Improved microbiological hydroxylation of sesquiterpenoids: semisynthesis, structural determination and biotransformation studies of cyclic sulfite eudesmane derivatives †

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Two new cyclic sulfite eudesmane derivatives have been investigated. Their (*R*) and (*S*) sulfur configuration and the structural arrangement of their "A" rings have been assigned by means of their ^{13}C and ^{1}H NMR chemical shifts and have been confirmed by single-crystal X-ray analyses. Microbial-transformation of these epimer cyclic sulfites and their dihydroxyeudesmane precursor have been studied using the hydroxylating fungus *Rhizopus nigricans*. Increased biocatalysis rates and considerable differences in the biotransformation of both cyclic sulfite eudesmanes have been found. Promising 8α,11-dihydroxy derivatives have been isolated from the (*S*)-diastereomer bioconversion.

Introduction

Biocatalysis is increasingly becoming an important tool for organic synthesis. Thus, biotransformations with whole cells are currently accepted as methods to synthesise many fine chemicals, to prepare chiral building blocks or to modify natural products with biological activities.[1] One of the most widespread enzymatic activities is the hydroxylation,[2] but it is, perhaps, the least-well understood. The main catalytic properties of these monooxygenase enzymes are their high degrees of regio-and stereoselectivity in the hydroxylation of non-activated carbon centres.[3,4]

Terpenes represent the largest family of natural products [5] comprising over 30000 defined structures.[6] In particular, sesquiterpene compounds with eudesmane skeletons are widely distributed in nature [7,8] and are biologically quite active,[9–13] having, for example, antimicrobial,[14] antifeedant,[15] cell-growth-inhibiting properties *etc*. Consequently, their synthesis has received particular attention in the past few decades,[16,17] in addition to the fact that these compounds are often the starting materials for the semisynthesis of other products.[18–20]

Microbial hydroxylation of terpenoids[21] has been used for the selective functionalization of many of these compounds and constitutes an important alternative to chemical methods, enabling the specific access to remote positions on the molecule under mild reaction conditions. Therefore, we have previously described several biotransformations of diverse eudesmane substrates by different filamentous fungi, and we have isolated metabolites in which we observed both regio- and stereoselective hydroxylation.[22–26] The main action of these microorganisms was directed to the isopropyl moiety, and some of these isolated metabolites have been used, by combination with chemical methods, to synthesise natural sesquiterpene derivatives such as 6α- and 6β-sesquiterpenolides.[23,24] These biocatalytic studies have also shown the influence on the bioconversion yields of the configuration of a hydroxy group at C-4 in eudesmane compounds.[23,26]

In recent years, new synthetic applications of chiral cyclic sulfites as the intermediates in stereoselective transformations of diols have been developed.[27,28] Their regioselective ring opening in nucleophilic substitution reactions with strong nucleophiles, their oxidation reaction to give cyclic sulfates as versatile intermediates in synthesis, or their applications in the resolution of diols are of particular interest.[29] Recently, a biocatalytic study was performed with eudesman-4α,6α-diyl-S-cyclic sulfite derivatives.[30] Their different reactivity was explained by biotransformation with *Rhizopus nigricans*.

In the present paper, the synthesis of two new diastereomer eudesman-4β,6β-diyl-S-cyclic sulfite derivatives has been investigated. The absolute configuration of the sulfur atom in this pair of compounds has been shown by the analysis of their experimental NMR chemical shifts and their $^{3}J_{HH}$ coupling constants. The half-boat conformation of the eudesmane "A" ring, due to the presence of the new sulfite cycle, has also been displayed. These results have been confirmed by X-ray crystallography. Finally, the different reactivity of both diastereomer sulfites in the biotransformation processes with *Rhizopus nigricans* (CECT 2672), a synonym of *Rhizopus stolonifer* (ATCC 10404, IMI 061269), has also been studied, and promising hydroxylated metabolites have been isolated.

Results and discussion

The cyclic sulfite eudesmane derivatives **1** and **2** were derived from the natural compound **3**, isolated from *Sideritis leucantha* Cav. subsp. *meridionalis*.[31] Hydrolysis of the acetoxy group on C-6 of **3**, and subsequent regioselective Jones' oxidation of the alcohol on C-1 gave the diol **4**. The diastereomer cyclic sulfites were synthesised by treatment of this diol (**4**) with thionyl chloride in methylene chloride, adding pyridine as base, to scavenge the hydrogen chloride released during the process (Scheme 1).[32] The reaction was carried out at diverse temperatures between −45 and +45 °C – the reflux temperature of the reaction mixture – and different relative ratios of sulfite diastereomers **1** and **2** were found (Fig. 1, Table 1). Thus, when the reaction temper-

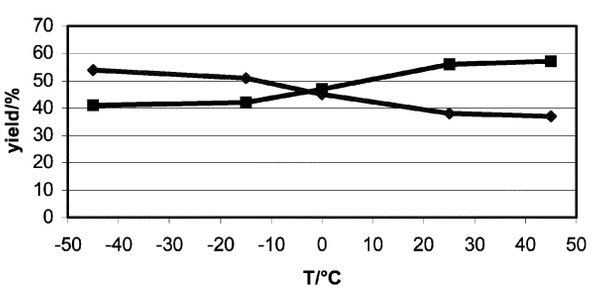

Fig. 1 Relative percentages of sulfites **1** (◆) and **2** (■) at different temperatures.

† Electronic supplementary information (ESI) available: NMR spectra. See http://www.rsc.org/suppdata/ob/b3/b301577g/

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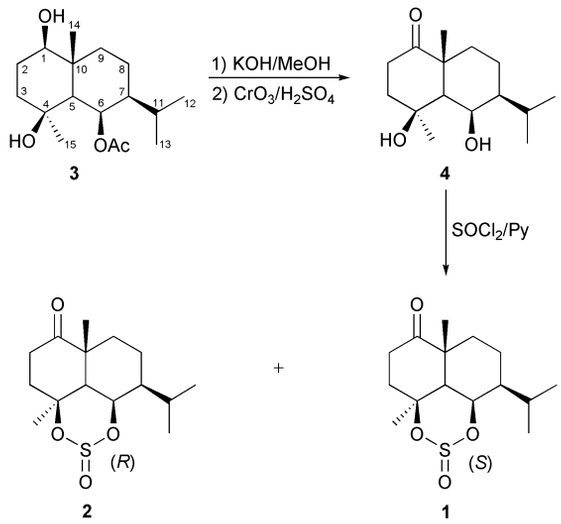
Table 1 Amounts and relative percentages of sulfites **1** and **2** at different temperatures

Temperature/°C	1	2
45	18.5 mg (37%)	28.5 mg (57%)
25	19.0 mg (38%)	28.0 mg (56%)
0	22.5 mg (45%)	23.5 mg (47%)
−15	25.5 mg (51%)	21.0 mg (42%)
−45	27.0 mg (54%)	20.5 mg (41%)



Scheme 1 Semisynthesis of cyclic sulfites **1** and **2**.

ature decreased, kinetic control of the process was favoured and the isomer **1** percentage increased. Conversely, the rise of the reaction temperature preferred thermodynamic control, and sulfite **2** yield was favoured.

The new six-membered sulfite rings occupied an almost identical chair conformation in both isomers (**1** and **2**), differing only by the S⟶O configuration. Consequently, they differed in their physical and chemical properties (reactivity, α_D, polarity). These new cycles included carbons 4, 5 and 6 of the eudesmane skeleton, and the oxygen atoms on C-4 and C-6, in a β-disposition. The absolute stereochemistry at the sulfur atoms and the preferential conformation of the "A" ring in these eudesmane derivatives (**1** and **2**) were determined by means of experimental ^{13}C and ^{1}H NMR data. The axial or equatorial relationship of the S ⟶ O bond in the sulfite cycles notably influenced the chemical shifts of the atoms in their neighbouring region. Thus, in the ^{1}H NMR spectrum of compound **1**, the 6α-H signal was at 5.55 ppm and the 15-Me signal at 1.87 ppm, while in the diastereomer **2** these protons appeared respectively at 4.96 and 1.74 ppm. According to these data, in compound **1** the S⟶O bond could occupy a *syn*-axial disposition, showing 1,3-diaxial interactions with the more deshielded 6α-H and 15-Me protons. Nevertheless, in compound **2** this S⟶O bond should form an approximate angle of 120° with the corresponding protons. On comparing the ^{13}C NMR spectra of these derivatives (**1** and **2**), we detected the main differences in the chemical shift of C-4, C-6 and C-15 (Table 2). The γ-*gauche* disposition in sulfite **1** and the γ-*anti* arrangement in sulfite **2**, between the S⟶O bond and C-4 and C-6, could explain the noticeable differences in these δ_C values for both diastereomers. Furthermore, the 1,3-diaxial interaction between the S⟶O bond and C-15, in sulfite **1**, illustrated the more deshielded chemical shift of this carbon. In summary, in compound **1** the S⟶O bond had a *syn*-axial disposition with regard to the cyclic sulfite ring, while in compound **2** it had an equatorial arrangement. Accordingly, we assigned the structure of 1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite for **1**, and 1-oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite for **2**.

The existence of the cyclic sulfite affects the conformation of the "A" ring in these eudesmane derivatives. On comparing the ^{1}H NMR spectra of both diastereomers and that of compound **4**, we observed significant differences in the chemical shifts and in the coupling constants of different signal protons of that ring. A clear difference was found for the 2β-H signals. Thus, in the spectrum of **4**, this proton appeared at δ 3.15, while in sulfites **1** and **2** the respective 2β-H signals were more shielded at δ 2.45 and 2.55, probably due to the absence of a 1,3-diaxial interaction with the oxygen atom on C-4. These discrepancies could be explained by a different conformation of the "A" ring in these compounds. Hence, in compound **4**, these experimental values agreed with a half-chair conformation to this ring, and with a half-boat conformation in both cyclic sulfite derivatives (**1** and **2**).

These hypotheses and the configuration of the sulfur atoms in the sulfite rings were confirmed by the X-ray data of both derivatives (Figs. 2 and 3).

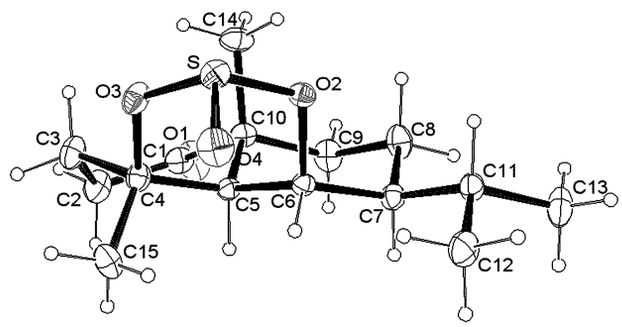


Fig. 2 X-Ray structure of compound **1**.

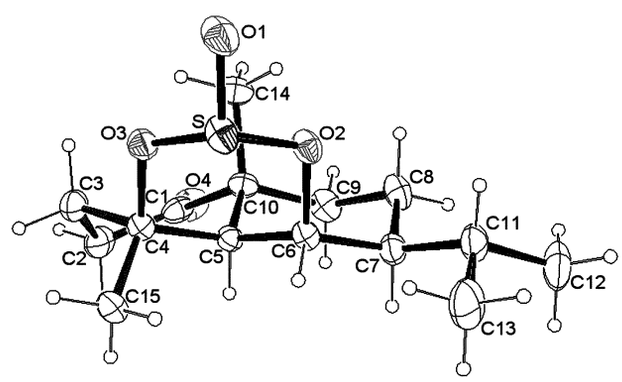


Fig. 3 X-Ray structure of compound **2**.

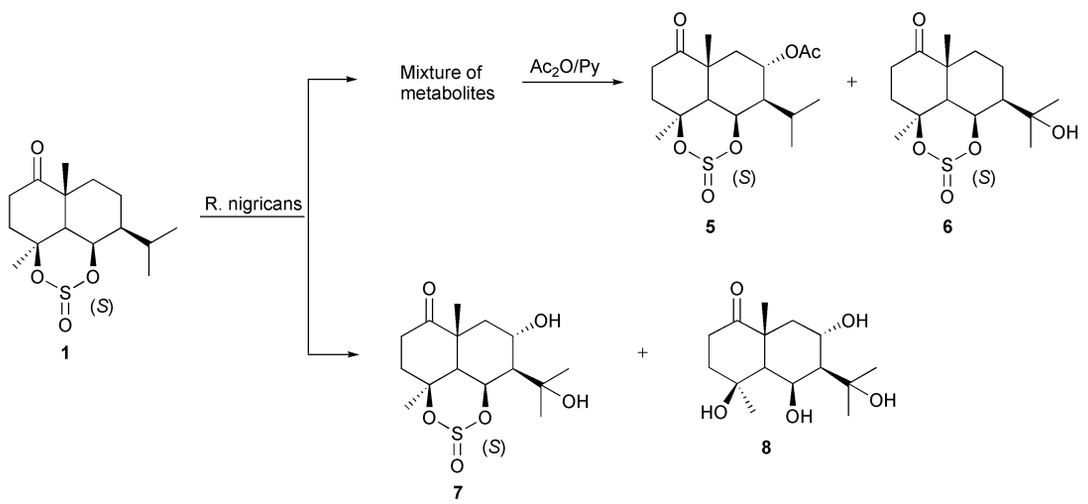
To explore the influence of the sulfur configuration in the biocatalysis reactions, shown in a previous work,[30] we studied the biotransformation of these sulfite derivatives with the fungus *R. nigricans*. Incubation of the (*S*)-sulfite derivative (**1**) was maintained for 36 h, resulting in total consumption of substrate (**1**). On the other hand, the biotransformation of the (*R*)-diastereomer (**2**) was carried out for 72 h (double time), leaving 20% of unaltered substrate (**2**). For a better comparison of the different reactivity of the cyclic sulfites diastereomers, the sulfite derivative **1** was also incubated for 72 h.

Biotransformation of cyclic sulfite **1** by *R. nigricans* for 36 h yielded a mixture of metabolites from which, after treatment with Ac_2O–Py, compound **5** (20%) and metabolite **6** (45%) were isolated. In addition, metabolite **7** (20%) and a very polar metabolite **8** (3%) were also found (Scheme 2).

The ^{1}H NMR spectrum of the first compound isolated (**5**) revealed a signal at δ 5.28 (ddd, $J_{8,7} = J_{8,9\alpha}$ 11.4, $J_{8,9\beta}$ 4.1) due to the geminal proton of an acetoxy group on C-8 with an equatorial arrangement. The ^{13}C NMR data confirmed the new acetoxy group position, and therefore, compound **5** was 8α-acetoxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite.

Table 2 ^{13}C NMR chemical shifts for compounds **1**, **2**, **4**, **5**, **6**, **7** and **8**

	Compounds						
	1	**2**	**4**	**5**	**6**	**7**	**8**
C-1	214.7	214.3	216.3	212.5	214.2	212.7	213.7
C-2	33.9	34.0	34.5	33.4	33.8	33.5	33.4
C-3	35.7	35.9	41.0	35.8	35.6	35.6	40.4
C-4	81.3	84.2	72.8	80.9	81.9	81.4	73.1
C-5	44.5	44.0	53.0	44.4	44.5	44.2	52.1
C-6	62.0	72.5	70.3	63.5	62.4	62.5	70.1
C-7	47.4	47.5	49.2	49.4	50.0	55.8	56.8
C-8	20.6	20.8	20.5	68.4	17.2	65.9	65.6
C-9	33.6	33.5	34.5	39.4	33.4	42.8	43.6
C-10	45.2	45.2	47.6	46.5	45.2	46.5	48.1
C-11	28.4	28.2	29.6	27.4	72.0	73.8	71.8
C-12	21.0	20.5	21.9	21.3	28.4	28.1	27.7
C-13	20.1	20.5	21.1	21.1	28.2	26.9	26.7
C-14	20.6	21.9	20.8	20.0	20.6	21.3	21.9
C-15	30.4	27.0	28.8	30.6	30.3	30.6	28.4
$CO(CH_3)$				170.1			
$CH_3(CO)$				20.1			



Scheme 2 Biotransformation of **1** by *Rhizopus nigricans* cultures.

Table 3 Yields of compounds in the biotransformation of **1** at 36 and 72 h

	Yield of compounds (%)			
t/h	**5**	**6**	**7**	**8**
36	20	45	20	3
72	15	29	20	23

The main metabolite isolated (**6**) had a new hydroxy group not acetylated, whose spectral data indicated a biohydroxylation on C-11. Consequently, metabolite (**6**) was 11-hydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite.

The comparison of the spectral data of metabolite **7** with those of compound **5** and metabolite **6** showed the presence of two new hydroxy groups at C-8 and C-11, which agreed with a structure of 8α,11-dihydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite for this metabolite (**7**).

The last metabolite isolated (**8**) was considerably more polar than the others, due to the absence of the sulfite group, and with spectral data similar to those of metabolite **7**. Hence, we deduced that metabolite **8** was 4β,6β,8α,11-tetrahydroxy-eudesman-1-one.

The same four compounds were isolated when the biotransformation of substrate **1** was maintained for 72 h. Nevertheless, the compound ratio presented substantial changes (Table 3). The whole percentage of 8α,11-dihydroxylated compounds was almost doubled (23% for 36 h and 43% for 72 h), while the yield of metabolites without sulfite group also increased.

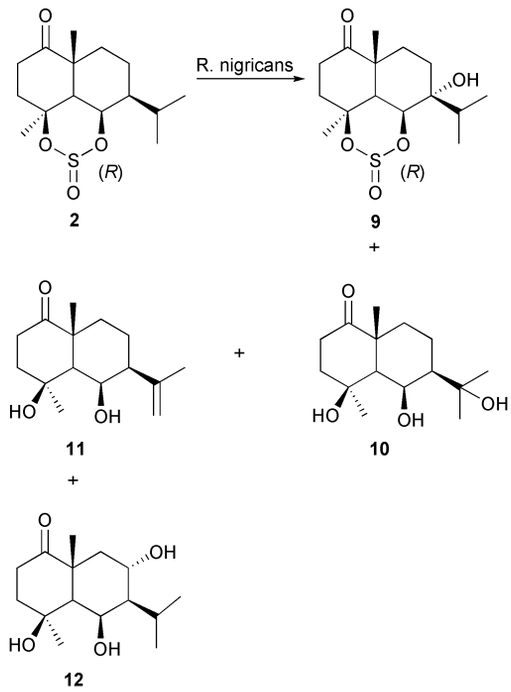
Biotransformation of the (*R*)-cyclic sulfite diastereomer (**2**) with *R. nigricans* for 72 h gave the metabolites **9** (5%), **10** (50%), **11** (2%) and **12** (10%), besides some 20% of unaltered substrate (**2**) (Scheme 3).

Metabolite **9** had an additional hydroxy group but without geminal proton. The ^{13}C NMR spectral data positioned this new hydroxy group at C-7. Consequently, metabolite (**9**) had the structure of 7α-hydroxy-1-oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite.

The spectral data of the main metabolite isolated (**10**) indicated the presence of a new hydroxy group and the loss of the cyclic sulfite group. This compound was a 4-*epi*-cryptomeridiol[33] derivative (4β,6β,11-trihydroxyeudesman-1-one), previously isolated from the incubation of different eudesmane derivatives with *R. nigricans*[23] and *Gliocladium roseum*.[26]

The spectral data of metabolite **11** indicated the presence of a carbon–carbon double bond placed between C-11 and C-12, probably formed by a dehydration reaction from an 11-hydroxy compound, and the absence of the sulfite group. Hence, this metabolite (**11**) was 4β,6β-dihydroxyeudesm-11-en-1-one.

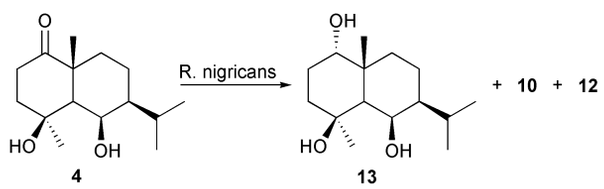
The most polar metabolite (**12**), which had lost the sulfite group, owned a new hydroxy group situated on C-8 in an equatorial arrangement, with a structure of 4β,6β,8α-trihydroxy-eudesman-1-one.



Scheme 3 Biotransformation of **2** by *Rhizopus nigricans* cultures.

To evaluate the positive influence of the cyclic sulfite groups in the biocatalysis processes, we also tested the eudesmane diol **4**.

Biotransformation of compound **4** by *R. nigricans* for 6 days yielded the metabolite **13** (20%), together with the compounds **10** (41%) and **12** (11%), previously isolated in the bioconversion of **2**, and 12% of the unaltered substrate (**4**) (Scheme 4).



Scheme 4 Biotransformation of **4** by *Rhizopus nigricans* cultures.

The first isolated metabolite (**13**) was the result of the stereoselective reduction of the carbonyl group at C-1 of substrate **4**. This reduction, which was achieved from the β face due to the steric hindrance, originated a (1*S*)-hydroxylated derivative, as is usual in enzymatic reductions.[34] Therefore, metabolite **13** was 1a,4b,6b-trihydroxyeudesmane.

In summary, the presence of a cyclic sulfite in these substrates boosts the rate of bioconversion. Also, significant differences in the reactivity of the two diastereomers (**1** and **2**) were found. The (*S*)-sulfite **1** was entirely biotransformed after 36 h and dihydroxylated derivatives were isolated from this incubation. On the other hand, 20% of the (*R*)-sulfite **2** was recovered after a double incubation period, and only compounds from a single microbial hydroxylation were detected. Also, the (*R*)-sulfite derivatives seem more easily to lose the cyclic sulfite in the bioconversion medium. The main actions of *R. nigricans* on this kind of substrate were directed towards the isopropyl moiety and C-8. From the bioconversion of the (*S*)-sulfite (**1**) products arising only from a C-8α and/or C-11 hydroxylations were detected, whereas, from the biotransformation of the (*R*)-sulfite (**2**), 7α-hydroxy and 11-ene derivatives were also found, but in limited proportion. Previously, 8α,11-dihydroxy derivatives have been isolated but with poor yields,[26] while, in the present work, some 23% after only 36 h and 43% after 72 h were achieved. The versatile reactivity of this sulfite cyclic group and the 8α,11-dihydroxy derivatives isolated from the biotransformation of the (*S*)-diastereomer (**1**) offers new attractive possibilities to synthesise natural product derivatives such as 8,12-eudesmanolides.

Experimental

General experimental procedures

Measurements of NMR spectra (300.13 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$, CD_3COCD_3 or CD_3OD (which also provided the lock signal) with BRUKER spectrometers (AM-300 and ARX-400). 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 C/H correlation (HMQC). IR spectra were recorded on a Nicolet 20SX FT-IR spectrometer. High-resolution mass spectra were made by LSIMS ionization mode with a MICROMASS AUTOSPEC-Q spectrometer. X-Ray data were collected on a BRUKER P4 diffractometer equipped with graphite-monochromator Mo-Kα radiation (l = 0.71073 Å). The crystal structures of compounds **1** and **2** were refined using the SHELXTL program package (ver. 6.10). Uncorrected melting points were determined using a Kofler (Reichter) apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 25 °C and are given in units of 10^{-1} deg cm^2 g^{-1}. Scharlau 60 silica gel (40–60 μm) was used for flash chromatography. CH_2Cl_2, $CHCl_3$, or hexane containing increasing amounts of Me_2CO, MeOH or 2-propanol 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 °C. The identity of compounds **3** and **4** was confirmed by direct comparison with the authentic samples (IR, MS, NMR, *etc.*).

Isolation of 6β-acetoxy-1β,4β-dihydroxyeudesmane (3)

6β-Acetoxy-1β,4β-dihydroxyeudesmane was isolated from *Sideritis leucantha* Cav. subsp. *meridionalis* (Font Quer) O. Socorro.[31]

Synthesis of 4β,6β-dihydroxyeudesman-1-one (4)

6β-Acetoxy-1β,4β-dihydroxyeudesmane (**3**, 1.90 g, 6.4 mmol) was dissolved into a MeOH–H_2O (70%) solution (120 mL) containing KOH (5%) (6 g, 0.11 mol) and refluxed for 1 h. The reaction mixture was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and evaporated to dryness. Next, the resulting residue was stirred in acetone (50 mL) at 0 °C and Jones' reagent was added dropwise until an orange–brown colour persisted (30 min), following the monooxidation by TLC. Methanol was then added and the reaction mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure. Chromatography on a silica-gel column yielded 4β,6β-dihydroxyeudesman-1-one[23] (**4**, 1.54 g, 95%).

Formation study of sulfite derivatives (1 and 2) at different temperatures

Diol **4** (50 mg, 0.2 mmol) was dissolved in dichloromethane (1.5 mL) and pyridine (0.5 mL) in each experiment. The mixture was stirred at the adequate temperature and thionyl chloride (0.07 mL, 1 mmol) was added. After 5 min, water (4 mL) was cautiously added dropwise. The mixture was extracted with CH_2Cl_2 (3 × 4 mL), and the combined extracts were washed with saturated aqueous $KHSO_4$ (2 × 10 mL), dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Column chromatography over silica gel using hexane and increasing amounts of isopropanol as eluents gave 1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (**1**) and 1-oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite (**2**) (yields in Table 1).

1-Oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (1). Colourless solid, mp 88–90 °C (from hexane); $[\alpha]_D^{25} = -97$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 2964, 1717, 1220, 1178 and 1150; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.95 and 0.97 (3 H each, d, *J* 6.5, 12-Me and 13-Me), 1.05 (1 H, dddd, $J_{7,8\beta}$ 12.5, $J_{7,11}$ 9.1, $J_{7,8\alpha}$ 3.7, $J_{7,6}$ 3.3, 7-H), 1.20 (1 H, ddd, $J_{9\alpha,9\beta} = J_{9\alpha,8\beta}$ 13.6, $J_{9\alpha,8\alpha}$ 3.7, 9α-H), 1.44 (3 H, s, 14-Me), 1.48 (1 H, d, $J_{5,6}$ 2.4, 5-H), 1.49 (1 H, dddd, $J_{8\beta,8\alpha}$ 13.9, $J_{8\beta,9\alpha}$ 13.6, $J_{8\beta,7}$ 12.5, $J_{8\beta,9\beta}$ 3.3, 8β-H), 1.66 (1 H, m, $J_{11,7}$ 9.1, $J_{11,12} = J_{11,13}$ 6.5, 11-H), 1.80 (1 H, dddd, $J_{8\alpha,8\beta}$ 13.9, $J_{8\alpha,7} = J_{8\alpha,9\alpha}$ 3.7, $J_{8\alpha,9\beta}$ 3.3, 8α-H), 1.87 (3 H, s, 15-Me), 2.00 (1 H, ddd, $J_{9\beta,9\alpha}$ 13.6, $J_{9\beta,8\beta} = J_{9\beta,8\alpha}$ 3.3, 9β-H), 2.45 (1 H, ddd, $J_{2\beta,2\alpha}$ 15.4, $J_{2\beta,3\alpha}$ 3.7, $J_{2\beta,3\beta}$ 2.5, 2β-H) and 5.55 (1 H, dd, $J_{6,7}$ 3.3, $J_{6,5}$ 2.4, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 323.1296 ([M + 23]$^+$. $C_{15}H_{24}O_4SNa$ requires 323.1293).

1-Oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite (2). Colourless solid, mp 113–115 °C (from hexane); $[\alpha]_D^{25} = -39$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 2959, 1711, 1222 and 1192; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.95 and 0.97 (3 H each, d, *J* 6.5, 12-Me and 13-Me), 1.09 (1 H, dddd, $J_{7,8\beta}$ 12.6, $J_{7,11}$ 9.1, $J_{7,8\alpha}$ 3.7, $J_{7,6}$ 3.3, 7-H), 1.21 (1H, ddd, $J_{9\alpha,9\beta} = J_{9\alpha,8\beta}$ 13.6, $J_{9\alpha,8\alpha}$ 3.7, 9α-H), 1.33 (1 H, d, $J_{5,6}$ 2.4, 5-H), 1.48 (3 H, s, 14-Me), 1.53 (1 H, dddd, $J_{8\beta,8\alpha} = J_{8\beta,9\alpha}$ 13.6, $J_{8\beta,7}$ 12.6, $J_{8\beta,9\beta}$ 3.3, 8β-H), 1.74 (3 H, s, 15-Me), 1.78 (1 H, dddd, $J_{8\alpha,8\beta}$ 13.6, $J_{8\alpha,7} = J_{8\alpha,9\alpha}$ 3.7, $J_{8\alpha,9\beta}$ 3.3, 8α-H), 1.96 (1 H, ddd, $J_{9\beta,9\alpha}$ 13.6, $J_{9\beta,8\beta} = J_{9\beta,8\alpha}$ 3.3, 9β-H), 2.55 (1 H, ddd, $J_{2\beta,2\alpha}$ 16.4, $J_{2\beta,3\alpha} = J_{2\beta,3\beta}$ 4.3, 2β-H) and 4.96 (1 H, dd, $J_{6,7}$ 3.3, $J_{6,5}$ 2.4, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 323.1292 ([M + 23]$^+$. $C_{15}H_{24}O_4SNa$ requires 323.1293).

Synthesis of sulfites derivatives (1 and 2)

For the biotransformation studies of these cyclic sulfite derivatives (**1** and **2**), their formation reaction was again carried out at 0 °C. For this, 4β,6β-dihydroxyeudesman-1-one (**4**, 1.20 g, 4.7 mmol) was dissolved in dichloromethane (40 mL) and pyridine (11 mL), and then, thionyl chloride (1.67 mL, 23.5 mmol) was added dropwise. After previously indicated treatment, (*S*)-cyclic sulfite **1** (638 mg, 45%) and (*R*)-cyclic sulfite **2** (666 mg, 47%) were isolated.

Crystal structure determination of compounds 1 and 2‡

Crystals of compounds **1** and **2** suitable for X-ray diffraction were obtained by slow evaporation of hexane solution into a methanol atmosphere.

Crystal data for 1. $C_{15}H_{24}O_4S$, M = 300.4, monoclinic, a = 8.519(1), b = 9.782(1), c = 18.631(2) Å, U = 1552.6(2) Å^3, T = 294(1) K, space group $P2_12_12_1$, Z = 4, D_{calc} = 1.285 Mg m^{-3}, $F(000)$ = 648, μ(Mo–Kα) = 0.219 mm^{-1}, 3332 reflections measured, 3129 unique ($R_{(int)}$ = 0.0349, R_σ = 0.0396) which were used in all calculations. R_1 = 0.0409 ($F_0 > 4\sigma(F_0)$) and 0.0535 (for all). Flack = −0.20 (9). The final $wR(F^2)$ was 0.1086.

Crystal data for 2. $C_{15}H_{24}O_4S$, M = 300.4, monoclinic, a = 9.321(2), b = 7.996(2), c = 11.132(2) Å, U = 796.2(3) Å^3, T = 294(1) K, space group $P2_1$, Z = 2, D_{calc} = 1.253 Mg m^{-3}, $F(000)$ = 324, μ(Mo-Kα) = 0.213 mm^{-1}, 3060 reflections measured, 3060 unique ($R_{(int)}$ = 0.0000, R_σ = 0.0330) which were used in all calculations. R_1 = 0.0672 ($F_0 > 4\sigma(F_0)$) and 0.1050 (for all). Flack = −0.18 (16). The final $wR(F^2)$ was 0.1730.

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 beef extract medium (BEM) 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 the substrates **1**, **2** and **4** (5–10%) in EtOH were added.

Biotransformation of (*S*)-cyclic sulfite 1 (36 h)

Substrate **1** (375 mg) was dissolved in EtOH (5 ml), distributed among 5 Erlenmeyer flask cultures of *R. nigricans* and incubated for 36 h, 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, which was chromatographed on a silica gel column to provide a mixture of metabolites (258 mg, 65%). From this, after treatment with Ac_2O–Py, 8α-acetoxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (**5**, 80 mg, 20% of overall yield) and 11-hydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (**6**, 178 mg, 45% of overall yield) were isolated, together with 8α,11-dihydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (**7**, 85 mg, 20%) and 4β,6β,8α,11-tetrahydroxyeudesman-1-one (**8**, 12 mg, 3%).

8α-Acetoxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (5). Colourless solid, mp 124–126 °C; $[\alpha]_D^{25} = 0$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 2961, 1717, 1244 and 1186; δ_H(300 MHz; $CDCl_3$; Me_4Si); 0.97 and 1.01 (3 H each, d, *J* 7.0, 12-Me and 13-Me), 1.51 (1 H, d, $J_{5,6}$ 2.9, 5-H), 1.55 (3 H, s, 14-Me), 1.87 (3 H, s, 15-Me), 2.02 (3 H, s, Me-acetoxyl group), 5.28 (1 H, ddd, $J_{8,7} = J_{8,9\alpha}$ 11.4, $J_{8,9\beta}$ 4.1, 8-H) and 5.67 (1 H, dd, $J_{6,7}$ 3.1, $J_{6,5}$ 2.9, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 381.1342 ([M + 23]$^+$. $C_{17}H_{26}O_6SNa$ requires 381.1348).

11-Hydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (6). Colourless solid, mp 119–121 °C; $[\alpha]_D^{25} = -96$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3516, 2960, 1718, 1220 and 1182; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.25 (1 H, ddd, $J_{9\alpha,9\beta} = J_{9\alpha,8\beta}$ 13.4, $J_{9\alpha,8\alpha}$ 4.3, 9α-H), 1.33 and 1.26 (3 H each, s, 12-Me and 13-Me), 1.42 (1 H, ddd, $J_{7,8\beta}$ 12.3, $J_{7,8\alpha}$ 4.3, $J_{7,6}$ 3.0, 7-H), 1.46 (3 H, s, 14-Me), 1.49 (1 H, d, $J_{5,6}$ 2.4, 5-H), 1.84 (1 H, dddd, $J_{8\beta,8\alpha} = J_{8\beta,9\alpha}$ 13.4, $J_{8\beta,7}$ 12.6, $J_{8\beta,9\beta}$ 3.3, 8β-H), 1.88 (3 H, s, 15-Me), 2.06 (1 H, ddd, $J_{9\beta,9\alpha}$ 13.4, $J_{9\beta,8\beta} = J_{9\beta,8\alpha}$ 3.3, 9β-H), 2.46 (1 H, ddd, $J_{2\beta,2\alpha}$ 16.2, $J_{2\beta,3\alpha} = J_{2\beta,3\beta}$ 2.9, 2β-H) and 5.79 (1 H, dd, $J_{6,7}$ 3.0, $J_{6,5}$ 2.4, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 339.1243 ([M + 23]$^+$. $C_{15}H_{24}O_5SNa$ requires 339.1242).

8α,11-Dihydroxy-1-oxoeudesman-4β,6β-diyl-S(*S*)-cyclic sulfite (7). Colourless solid, mp 168–170 °C; $[\alpha]_D^{25} = -60$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3310, 2971, 1714, 1214 and 1186; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.41 and 1.33 (3 H each, s, 12-Me and 13-Me), 1.49 (3 H, s, 14-Me), 1.88 (3 H, s, 15-Me), 4,37 (1 H, ddd, $J_{8,9\alpha} = J_{8\beta,7}$ 11.2, $J_{8,9\beta}$ 4.1, 8β-H) and 5.67 (1 H, dd, $J_{6,7} = J_{6,5}$ 2.9, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 355.1191 ([M + 23]$^+$. $C_{15}H_{24}O_6SNa$ requires 355.1191).

4β,6β,8α,11-Tetrahydroxyeudesman-1-one (8). Colourless solid, mp 194–196 °C; $[\alpha]_D^{25} = +65$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3403, 2924, 1712, 1167 and 1072; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.18 (1 H, d, $J_{5,6}$ 2.3, 5-H), 1.18 (1 H, dd, $J_{7,8}$ 13.9, $J_{7,6}$ 2.9, 7-H), 1.27 and 1.25 (3 H each, s, 12-Me and 13-Me), 1.35 (3 H, s, 15-Me), 1.54 (3 H, s, 14-Me), 1.68 (1 H, ddd, $J_{3\alpha,2\beta} = J_{3\alpha,3\beta}$ 13.9, $J_{3\alpha,2\alpha}$ 4.3, 3α-H), 3.01 (1 H, ddd, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha}$ 13.9,

‡ CCDC reference numbers 203948 and 203949. See http://www.rsc.org/suppdata/ob/b3/b301577g/ for crystallographic data in .cif or other electronic format.

Table 4 ^{13}C NMR chemical shifts for compounds **9**–**13**

	Compounds				
	9	**10**	**11**	**12**	**13**
C-1	214.2	216.3	216.2	215.0	76.0
C-2	34.0	34.3	34.6	34.1	25.2
C-3	36.1	41.0	40.5	41.1	36.5
C-4	85.0	72.7	72.0	72.7	73.7
C-5	39.7	52.8	52.7	52.9	46.0
C-6	74.4	69.3	68.6	72.9	69.2
C-7	73.4	49.1	49.5	54.1	49.8
C-8	27.6	16.8	21.2	66.2	20.7
C-9	28.4	34.6	34.2	44.6	35.1
C-10	44.7	47.5	47.7	48.1	37.8
C-11	32.4	74.1	145.7	27.9	28.8
C-12	15.6	29.3	112.8	22.9	22.1
C-13	15.6	28.9	23.0	21.2	21.2
C-14	21.5	21.9	22.0	20.6	20.9
C-15	26.9	29.5	29.4	29.6	30.4

$J_{2\beta,3\beta}$ 6.4, 2β-H), 4.29 (1 H, ddd, $J_{8,9\alpha} = J_{8\beta,7}$ 11.2, $J_{8,9\beta}$ 4.3, 8β-H) and 4.54 (1 H, dd, $J_{6,7}$ 2.9, $J_{6,5}$ 2.3, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 2; *m/z* (HRLSIMS) 309.1679 ([M + 23]$^+$. $C_{15}H_{26}O_5Na$ requires 309.1678).

Biotransformation of (*S*)-cyclic sulfite 1 (72 h)

Substrate **1** (150 mg) was also dissolved in EtOH (3 ml), distributed among 3 Erlenmeyer flask cultures of *R. nigricans* and incubated for 72 h, after which the cultures were processed as indicated above to give a mixture of metabolites (137 mg, 44%) from which, after treatment with Ac_2O–Py, **5** (24 mg, 15% of overall yield) and **6** (46 mg, 29% of overall yield) were isolated. In addition, **7** (33 mg, 20%) and **8** (33 mg, 23%) were also found.

Biotransformation of (*R*)-cyclic sulfite 2

Substrate **2** (375 mg) was dissolved in EtOH (5 ml), distributed among 5 Erlenmeyer flask cultures of *R. nigricans* and incubated for 72 h, after which the cultures were processed as previously indicated for the biotransformation of the substrate **1**, to give a mixture (333 mg). The mixture was chromatographed on a silica gel column to provide the starting material **2** (75 mg, 20%), 7α-hydroxy-1-oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite (**9**, 20 mg, 5%), 4β,6β,11-trihydroxyeudesman-1-one (**10**, 169 mg, 50%),[23] 4β,6β-dihydroxyeudesm-11-en-1-one (**11**, 6 mg, 2%) and 4β,6β,8α-trihydroxyeudesman-1-one (**12**, 34 mg, 10%).

7α-Hydroxy-1-oxoeudesman-4β,6β-diyl-S(*R*)-cyclic sulfite (9). Colourless solid, mp 141–143 °C; $[\alpha]_D^{25} = -34$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3512, 2966, 1712, 1230 and 1154; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.93 and 0.94 (3 H each, d, *J* 6.8, 12-Me and 13-Me), 1.48 (3 H, s, 14-Me), 1.74 (3 H, s, 15-Me) and 4.58 (1 H, d, *J* 2.4, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 4; *m/z* (HRLSIMS) 339.1243 ([M + 23]$^+$. $C_{15}H_{24}O_5SNa$ requires 339.1242).

4β,6β-Dihydroxyeudesm-11-en-1-one (11). Colourless syrup; $[\alpha]_D^{25} = +43$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3397, 3085, 1706, 1647, 1187 and 1130; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.33 (3 H, s, 15-Me), 1.60 (3 H, s, 14-Me), 1.79 (3 H, br s, 13-Me), 3.17 (1 H, ddd, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha}$ 14.0, $J_{2\beta,3\beta}$ 6.7, 2β-H), 4.35 (1 H, br s, 6-H), 5.03 (1 H, m, 12-H) and 4.87 (1 H, br s, 12′-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 4; *m/z* (HRLSIMS) 275.1622 ([M + 23]$^+$. $C_{15}H_{24}O_3Na$ requires 275.1623).

4β,6β,8α-Trihydroxyeudesman-1-one (12). Colourless solid, mp 158–160 °C; $[\alpha]_D^{25} = +53$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3396, 2924, 1691, 1135 and 1113; δ_H(300 MHz; $CDCl_3$; Me_4Si); 1.14 and 1.12 (3 H each, d, *J* 7.0, 12-Me and 13-Me), 1.39 (3 H, s, 15-Me), 1.64 (3 H, s, 14-Me), 3.13 (1 H, ddd, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha}$ 14.0, $J_{2\beta,3\beta}$ 6.5, 2β-H), 4.19 (1 H, ddd, $J_{8,7} = J_{8,9\alpha}$ 11.1, $J_{8,9\beta}$ 4.3, 8-H) and 4.64 (1 H, br s, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 4; *m/z* (HRLSIMS) 293.1727 ([M + 23]$^+$. $C_{15}H_{26}O_4Na$ requires 293.1729).

Biotransformation of diol 4

Substrate **4** (90 mg) was also dissolved in EtOH (1 ml), added in 1 Erlenmeyer flask cultures of *R. nigricans* and incubated for 6 days, after which the cultures were processed as indicated above to give the unaltered substrate (**4**, 11 mg, 12%), 6β-acetoxy-1α,4β-dihydroxyeudesmane (**13**, 18 mg, 20%) and compounds **10** (39 mg, 41%) and **12** (11 mg, 11%), previously isolated in the bioconversion of **2**.

1a,4b,6b-trihydroxyeudesmane (13). Colourless syrup; $[\alpha]_D^{25} = +32$ (*c* 1 in $CHCl_3$); ν_{max} (NaCl)/cm^{-1} 3418, 2923, 1136 and 1022; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.94 and 0.97 (3 H each, d, *J* 6.7, 12-Me and 13-Me), 1.30 (3 H each, s, 14-Me and 15-Me), 3.33 (1 H, br s, 1-H) and 4.57 (1 H, m, 6-H); δ_C(75.74 MHz; $CDCl_3$; Me_4Si) Table 4; *m/z* (HRLSIMS) 279.1934 ([M + 23]$^+$. $C_{15}H_{28}O_3Na$ requires 279.1936).

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