PERGAMON

Phytochemistry 58 (2001) 891–895

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Biotransformation of 4β-hydroxyeudesmane-1,6-dione by *Gliocladium roseum* and *Exserohilum halodes*

Andrés García-Granados[a,*], María C. Gutiérrez[a], Francisco Rivas[a], José M. Arias[b]

[a]*Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*
[b]*Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*

Received 8 June 2001; received in revised form 24 July 2001

Abstract

Biotransformation of sesquiterpene 4β-hydroxyeudesmane-1,6-dione by the filamentous fungi *Gliocladium roseum* and *Exserohilum halodes* was achieved. With *Exserohilum halodes*, only one metabolite was obtained, as a result of the regio- and stereoselective reduction of the keto group at C-1, which is difficult to achieve by chemical means. Five metabolites were produced with *Gliocladium roseum*, three of which, the 7α-hydroxylated, the 7α,11- and the 1α,8α-dihydroxylated derivatives, have not previously been reported. The hydroxylation at C-11 is the main action of this microorganism. These 11-hydroxylated compounds can be chemically transformed into 6β,12-eudesmanolides. © 2001 Published by Elsevier Science Ltd.

Keywords: Biotransformation; Sesquiterpene; Eudesmane; Hydroxylation; *Gliocladium roseum*; *Exserohilum halodes*

1. Introduction

Gliocladium roseum (CECT 2733), the anamorphic form of *Nectria ochroleuca* (IMI 40022), is a filamentous fungus that had been used previously to hydroxylate the sesquiterpene patchoulol (Becher et al., 1978) as well as to biotransform a derivative of the diterpene varodiol in order to produce an *ent*-ambrox® derivative (García-Granados et al., 1999). *Exserohilum halodes* (CECT 2716) is a synonym of *Exserohilum rostratum*, the anamorphic form of *Setosphaeria rostrata* (IMI 76563).

Eudesmane sesquiterpenes are common in nature (Fischer et al., 1979; Fraga, 2000) and they possess significant biological properties (Rodríguez et al., 1976; Picman, 1986; Robles et al., 1995; Marles et al., 1995; Fraga, 1997; Schmidt, 1999). Several biotransformations of eudesmane compounds have been accomplished in the last decade (Amate et al., 1991; Atta-ur-Rahman et al., 1994; Shimizu et al., 1994; García et al., 1995; El-Sharkawy et al., 1996; Miyazawa et al., 1997; García-Granados et al., 1998, 2000a,b).

In previous papers (García-Granados et al., 1991, 1993) we reported the incubations of several 4β-hydroxyeudesmane compounds by the filamentous fungi *Curvularia lunata* (ATCC 12017) and *Rhizopus nigricans* (ATCC 10404). The action of these microorganisms was directed mainly to the isopropyl moiety, *Curvularia lunata* to C-12 and *Rhizopus nigricans* to C-11. In both cases, the best results were obtained on the 1,6-diketo derivative. Consequently, we have chosen this substrate to carry out several biotransformations with the microorganisms *Exserohilum halodes* and *Gliocladium roseum*.

2. Results and discussion

4β-Hydroxyeudesmane-1,6-dione **(1)** was obtained from a natural compound **(2)** isolated from *Sideritis varoi* subsp. *cuatrecasasii* (García-Granados et al., 1985), a synonym of *Sideritis leucantha* Cav. subsp. *meridionalis* (Font Quer) O. Socorro (Socorro, 2001). Deacetylation of **2** gave the trihydroxyl derivative **3**, which was oxidized to give the 1,6-diketo derivative **1**.

The incubation of **1** with *Exserohilum halodes* for 13 days produced only a limited quantity (15%) of meta-

* Corresponding author. Tel./Fax: +34-958-243364.
E-mail address: agarcia@ugr.es (A. García-Granados).

0031-9422/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0031-9422(01)00340-5

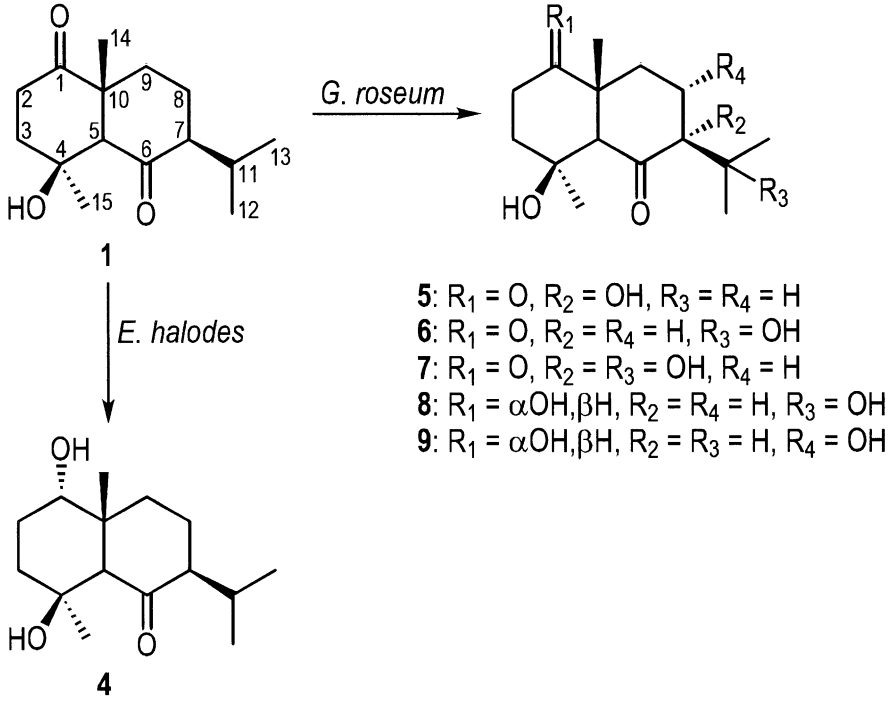

Fig. 1. Bioconversion of sesquiterpene 4β-hydroxyeudesmane-1,6-dione by *Gliocladium roseum* and *Exserohilum halodes*.

bolite **4**. This metabolite (**4**) was the result of the regio- and stereoselective reduction of the keto group at C-1 of substrate **1** from the β face giving a (1*S*)-hydroxyl derivative, which, due to the steric hindrance, is difficult to achieve by chemical means. The *S* configuration at C-1 could easily be deduced from the signal in the ^{1}H NMR spectrum (δ 3.52, 1H, *dd*, $J_{1,2\beta} = J_{1,2\alpha} = 3.0$ Hz), with coupling constants corresponding to an equatorial proton. This metabolite previously appeared in the incubation of substrate **1** with the microorganism *Curvularia lunata* (García-Granados et al., 1991).

Substrate **1** was more efficiently biotransformed by *Gliocladium roseum*. Incubation of this compound for 5 days yielded the metabolites **5** (2%), **6** (27%), **7** (3%), **8** (58%) and **9** (5%).

Metabolite **5** had a molecular formula of $C_{15}H_{24}O_4$, which indicated the presence of an additional hydroxyl group in the molecule. The ^{13}C NMR spectrum revealed β-effects on C-8 and C-11 and γ-effects on C-5, C-9, C-12 and C-13, positioning the new hydroxyl group at C-7. Consequently, metabolite **5** was 4β,7α-dihydroxyeudesmane-1,6-dione.

Metabolite **6** had the same molecular formula as the above metabolite (**5**), and its spectral data suggested a new hydroxyl group at C-11. This metabolite (**6**) was previously obtained in the incubation of substrate **1** with *Rhizopus nigricans* (García-Granados et al., 1993).

The molecular formula ($C_{15}H_{24}O_5$) of the third metabolite (**7**) was in agreement with the presence of two new hydroxyl groups. These functional groups were positioned at C-7 and C-11, by comparison of the spectral data of this metabolite (**7**) with those of metabolites **5** and **6**. Hence, metabolite **7** was 4β,7α,11-trihydroxyeudesmane-1,6-dione.

The main metabolite isolated (**8**) showed spectral data that, by comparison with those of metabolites **4** and **6**, indicated that *Gliocladium roseum*, besides the introduction of a hydroxyl group at C-11, also had reduced the carbonyl group at C-1 of the molecule, producing an *S*-alcohol. This metabolite (**8**) also resulted from the incubation of substrate **1** with *Rhizopus nigricans* (García-Granados et al., 1993).

The last metabolite isolated (**9**) had the same molecular formula ($C_{15}H_{26}O_4$) as metabolite **8**. Its ^{1}H NMR spectrum revealed two signals of geminal protons to hydroxyl groups at δ 3.55 (1H, *dd*, $J_{1,2\beta} = J_{1,2\alpha} = 2.9$ Hz), characteristic of a 1α-hydroxyl group, and at δ 4.04 (1H, *ddd*, $J_{8,9\alpha} = 10.8$, $J_{8,7} = 10.1$, $J_{8,9\beta} = 5.0$ Hz) due to an axial proton. The position of the new function was determined by the β-effects found on the adjacent carbons (C-7 and C-9). Therefore, metabolite **9** was 1α,4β,8α-trihydroxyeudesman-6-one.

3. Conclusions

Several conclusions can be drawn from the above biotransformation results. On the one hand, bioconversion of substrate (**1**) by *Exserohilum halodes* is more limited, reducing only the carbonyl group at C-1, producing an *S*-alcohol, as is usual in enzymatic reductions (Sih and Rosazza, 1976). On the other hand, *Gliocladium*

roseum achieved the complete bioconversion of substrate (**1**), producing mainly 11-hydroxyl derivatives (88%). These 11-hydroxylated compounds can be chemically transformed into 12-hydroxyl derivatives, precursors of 6β,12-eudesmanolides (García-Granados et al., 1993). The main action achieved on the substrate (**1**) by *Gliocladium roseum* is similar to the carried out by *Rhizopus nigricans* (García-Granados et al., 1993), although the first one, also produced 7α- and 8α-hydroxylated derivatives. All the microorganisms tested on the substrate (**1**) have reduced only the carbonyl group at C-1, while the one situated at C-6 remained unaltered (García-Granados et al., 1991, 1993).

In summary *Exserohilum halodes* produced only one known compound of difficult access by chemical means while *Gliocladium roseum* originated five compounds, three of which, have not previously been reported.

4. Experimental

Measurements of NMR spectra (300.13 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal) in a Bruker AM-300 spectrometer. The assignments of ^{13}C chemical shifts (Table 1) were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. IR spectra were recorded on a Nicolet 20SX FT–IR spectrometer. High-resolution mass spectra were made by LSIMS (FAB) ionization mode in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Melting points (uncorr.) were determined using a Kofler (Reichter) apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 25°. Silica gel Scharlau 60 (40–60 μm) was used for flash chromatography. CH_2Cl_2 or $CHCl_3$ containing increasing amounts of Me_2CO were used as eluents. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with H_2SO_4–AcOH, followed by heating to 120°. The identity of compound **2** was confirmed by direct comparison with an authentic sample (IR, MS, NMR, etc.).

4.1. Isolation of 6β-acetoxy-1β,4β-dihydroxyeudesmane (*2*)

6β-Acetoxy-1β,4β-dihydroxyeudesmane was isolated from *Sideritis varoi* subsp. *cuatrecasasii* (García-Granados et al., 1985), a synonym of *Sideritis leucantha* Cav. subsp. *meridionalis* (Font Quer) O. Socorro (Socorro, 2001).

4.2. Deacetylation of *2*

6β-Acetoxy-1β,4β-dihydroxyeudesmane (**2**, 1.20 g) was dissolved in MeOH/H_2O (70%) (70 ml) containing KOH (5%) (3.5 g) and refluxed for 1 h. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 1β,4β,6β-trihydroxyeudesmane (**3**, 952 mg, 92%) (García-Granados et al., 1991).

4.3. Oxidation of *3*

Jones' reagent was added dropwise to a stirred solution of 1β,4β,6β-trihydroxyeudesmane (**3**, 905 mg) in acetone at room temp. until an orange-brown colour persisted (30 min), following the oxidation by TLC. Methanol was then added and the reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 4β-hydroxyeudesmane-1,6-dione (**1**, 815 mg, 91%) (García-Granados et al., 1991). Syrup; $[\alpha]_D = +67°$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3527, 1712 and 1705 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 0.87 and 0.91 (3H each, *d*, $J = 6.6$ Hz, 3H-12 and 3H-13), 1.24 and 1.25 (3H each, *s*, 3H-14 and 3H-15), 2.58 (1H, *s*, H-5) and 3.07 (1H, *ddd*, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha} = 14.2$, $J_{2\beta,3\beta} = 6.0$ Hz, H-2β).

4.4. Organism, media and culture conditions

Gliocladium roseum CECT 2733 and *Exserohilum halodes* CECT 2716 were obtained from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain, and was kept in YEPGA medium containing yeast extract (1%), peptone (1%), glucose (2%) and agar (2%) in H_2O at pH 5. In all transformation experiments a medium of peptone (0.1%), yeast extract (0.1%), beef extract (0.1%) and glucose (0.5%) in H_2O at pH 5.7 was used. Erlenmeyer flasks (250 ml) containing 80 ml of medium were inoculated with a dense suspension of the corresponding microorganism. The cultures were incubated by shaking (150 rpm) at 28° for 6 days, after which, substrate 1 (5–10%) in EtOH was added.

4.5. Biotransformation of substrate *1* with Exserohilum halodes

Substrate **1** (124 mg) was dissolved in EtOH (2 ml), distributed between 2 Erlenmeyer flask cultures and incubated for 13 days, after which the cultures were filtered and pooled; the cells were washed thoroughly with water and the liquid was satd. with NaCl and extracted twice with CH_2Cl_2. Both extracts were pooled, dried with anhydrous Na_2SO_4, and evaporated at 40° in vacuum to give a mixture of compounds (102 mg). This mixture was chromatographed on a silica gel column to obtain 83 mg of starting material **1** and 19 mg (15%) of 1α,4β-dihydroxyeudesman-1-one (**4**) (García-Granados

et al., 1991). Syrup; $[\alpha]_D = +21$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3444 and 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 and 0.88 (3H each, *d*, $J = 6.6$ Hz, 3H-12 and 3H-13), 0.98 (3H, *s*, 3H-14), 1.18 (3H, *s*, 3H-15), 2.64 (1H, *s*, H-5) and 3.52 (1H, *dd*, $J_{1,2\beta} = J_{1,2\alpha} = 3.0$ Hz, H-1).

4.6. Biotransformation of substrate *1* with Gliocladium roseum

670 mg of substrate **1** were dissolved in EtOH (9 ml), distributed between 9 Erlenmeyer flask cultures and incubated for 5 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1** with *Exserohilum halodes*. The resulting mixture (667 mg) was chromatographed on a silica gel column to obtain 11 mg (2%) of 4β,7α-dihydroxyeudesmane-1,6-dione (**5**); syrup; $[\alpha]_D = +91^\circ$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3514 and 1701 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.92 and 0.94 (3H each, *d*, $J = 6.8$ Hz, 3H-12 and 3H-13), 1.24 and 1.25 (3H each, s, 3H-14 and 3H-15), 3.08 (1H, *ddd*, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha} = 14,3$, $J_{2\beta,3\beta} = 6.1$ Hz, H-2β) and 3.54 (1H, *s*, H-5); HRLSIMS, m/z: $[M+Na]^+$ 291.1565, ($C_{15}H_{24}O_4Na$, 291.1572, PPM +2.4); 190 mg (27%) of 4β,11-dihydroxyeudesmane-1,6-dione (**6**) (García-Granados et al., 1993); syrup; $[\alpha]_D = +61^\circ$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3527 and 1700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.18 and 1.21 (3H each, *s*, 3H-12 and 3H-13), 1.23 and 1.24 (3H each, *s*, 3H-14 and 3H-15), 2.39 (1H, *dd*, $J_{7,8\beta} = 12.2$, $J_{7,8\alpha} = 7.3$ Hz, H-7), 2.55 (1H, *s*, H-5) and 3.03 (1H, *ddd*, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha} = 14.2$, $J_{2\beta,3\beta} = 6.0$ Hz, H-2β); 20 mg (3%) of 4β,7α,11-trihydroxyeudesmane-1,6-dione (**7**); syrup; $[\alpha]_D = +89^\circ$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3498 and 1700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 and 1.26 (3H each, *s*, 3H-14 and 3H-15), 1.30 and 1.44 (3H each, *s*, 3H-12 and 3H-13), 1.70 (1H, *ddd*, $J_{3\alpha,3\beta} = J_{3\alpha,2\beta} = 14.3$, $J_{3\alpha,2\alpha} = 4.5$ Hz, H-3α), 2.04 (1H, *ddd*, $J_{3\beta,3\alpha} = 14.3$, $J_{3\beta,2\beta} = 5.9$, $J_{3\beta,2\alpha} = 2.8$ Hz, H-3β), 2.15 (1H, *ddd*, $J_{2\alpha,2\beta} = 14.3$, $J_{2\alpha,3\alpha} = 4.5$, $J_{2\alpha,3\beta} = 2.8$ Hz, H-2α), 3.07 (1H, *ddd*, $J_{2\beta,2\alpha} = J_{2\beta,3\alpha} = 14.3$, $J_{2\beta,3\beta} = 5.9$ Hz, H-2β) and 3.45 (1H, *s*, H-5); HRLSIMS, m/z: $[M+Na]^+$ 307.1527, ($C_{15}H_{24}O_5Na$, 307.1521, PPM-1.9); 414 mg (58%) of 1α,4β,11-trihydroxyeudesman-6-one (**8**) (García-Granados et al., 1993); syrup; $[\alpha]_D = +50^\circ$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3443 and 1684 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00 (3H, *s*, 3H-14), 1.19 (3H, *s*, 3H-15), 1.18 and 1.23 (3H each, *s*, 3H-12 and 3H-13), 2.41 (1H, *ddd*, $J_{7,8\beta} = 12.7$, $J_{7,8\alpha} = 6.3$, $J_{7,5} = 1.1$ Hz, H-7), 2.64 (1H, *bs*, H-5) and 3.51 (1H, *dd*, $J_{1,2\beta} = J_{1,2\alpha} = 2.8$ Hz, H-1); and 32 mg (5%) of 1α,4β,8α-trihydroxyeudesman-6-one (**9**); syrup; $[\alpha]_D = +100^\circ$ ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$): 3412 and 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.96 (3H, *s*, 3H-14), 1.06 and 1.10 (3H each, *d*, $J = 7.1$ Hz, 3H-12 and 3H-13), 1.22 (3H, *s*, 3H-15), 2.62 (1H, *s*, H-5), 3.55 (1H, *dd*, $J_{1,2\beta} = J_{1,2\alpha} = 2.9$ Hz, H-1β), and 4.04 (1H, *ddd*, $J_{8,9\alpha} = 10.8$, $J_{8,7} = 10.1$, $J_{8,9\beta} = 5.0$ Hz, H-8); HRLSIMS, *m/z*: $[M+Na]^+$ 293.1727, ($C_{15}H_{26}O_4Na$, 293.1729, PPM +0.7).

Table 1
^{13}C NMR chemical shifts for compounds **1**, **4**, **5**, **6**, **7**, **8** and **9**

	Compounds						
	1	**4**	**5**	**6**	**7**	**8**	**9**
C-1	213.8	73.6	213.5	213.1	213.0	73.3	73.2
C-2	33.1	25.3	33.9	32.7	33.8	25.3	24.9
C-3	39.6	33.8	39.7	39.5	39.5	32.5	32.5
C-4	69.6	70.1	69.9	69.5	69.8	70.1	69.9
C-5	63.3	57.1	57.2	63.5	58.1	57.7	56.3
C-6	215.0	218.5	214.2	217.2	216.4	221.2	214.1
C-7	57.7	58.1	80.2	60.0	79.8	60.3	64.5
C-8	25.7	26.5	32.2	24.9	29.0	25.7	69.9
C-9	33.9	35.1	28.9	33.7	29.3	34.7	45.4
C-10	53.8	45.9	53.7	53.7	52.6	46.2	40.5
C-11	26.2	26.1	31.5	71.0	74.9	71.1	25.0
C-12	21.4	21.6	17.0	28.5	25.1	28.7	20.4
C-13	18.7	18.8	16.6	26.0	24.0	26.1	19.6
C-14	19.7	19.5	19.1	19.5	19.2	19.4	19.5
C-15	29.3	30.4	29.1	29.2	29.1	30.3	30.2

Acknowledgements

This work was financially supported by grants from the Comisión Interministerial de Ciencia y Tecnología (PM98-0213) and the Consejería de Educación y Ciencia de la Junta de Andalucía (FQM 0139). We thank Laura López (CECT) for her assistance with the taxonomy of the microorganisms used in this work and David Nesbitt for improving the use of English in the manuscript.

References

Amate, Y., García-Granados, A., Martínez, A., Sáenz de Buruaga, A., Bretón, J.L., Onorato, M.E., Arias, J.M., 1991. Biotransformation of 6β-eudesmanolides functionalized at C-3 with *Curvularia lunata* and *Rhizopus nigricans* cultures. Tetrahedron 47, 5811–5818.

Atta-ur-Rahman, Choudhary, M.I., Ata, A., Alam, M., Farooq, A., Perveen, S., Shekhani, M.S., 1994. Microbial transformations of 7α-hydroxyfrullanolide. J. Nat. Prod. 57, 1251–1255.

Becher, E., Schuep, W., Matzinger, P.K., Ehret, C., Teissiere, P.J., Maruyama, H., Suhara, Y., Ito, S., Ogawa, M., 1978. Hydroxypatchoulol and its derivatives. Ger. Offen. 2,739,449 (Cl. C07C35/22) 23 March 1978, Brit. Appl. 76/36,397, 02 Sept 1976; pp. 67.

El-Sharkawy, S., Zaghloul, A.M., Badria, F.A., Matooq, G.T., 1996. Biotransformation of α-santonin. Mansoura J. Pharm. 12, 99–109.

Fischer, N.H., Olivier, E.J., Fischer, H.D., 1979. The biogenesis and chemistry of sesquiterpene lactones. In: Herz, W., Grisebach, H., Kirby, G.W. (Eds.), Fortschritt der Chemie organischer Naturstoffe, Vol. 38. Springer-Verlag, New York, pp. 47–390.

Fraga, B.M., 1997. Natural sesquiterpenoids. Nat. Prod. Rep. 14, 145–162.

Fraga, B.M., 2000. Natural sesquiterpenoids. Nat. Prod. Rep. 17, 483–504.

García, Y., García-Granados, A., Martínez, A., Parra, A., Rivas, F., Arias, J.M., 1995. Microbial transformations of 6α- and 6β-eudesmanolides by *Rhizopus nigricans* cultures. J. Nat. Prod. 58, 1498–1507.

García-Granados, A., Molina, A., Sáenz de Buruaga, A., Sáenz de Buruaga, J.M., 1985. Sesquiterpenes from two subspecies of *Sideritis varoi*. Phytochemistry 24, 97–101.

García-Granados, A., Martínez, A., Onorato, M.E., Rivas, F., Arias, J.M., 1991. Chemical-microbiological synthesis of 6β-eudesmanolides by *Curvularia lunata* cultures from eudesmanes with functions at C-1 and C-6. Tetrahedron 47, 91–102.

García-Granados, A., Martínez, A., Parra, A., Rivas, F., Onorato, M.E., Arias, J.M., 1993. Chemical-microbiological synthesis of 6β-eudesmanolides from 11-hydroxyl derivatives obtained by *Rhizopus nigricans* cultures: synthesis of 6β-dendroserins. Tetrahedron 49, 1091–1102.

García-Granados, A., Parra, A., Simeó, Y., Extremera, A.L., 1998. Chemical, enzymatic and microbiological synthesis of 8,12-eudesmanolides: synthesis of sivasinolide and yomogin analogs. Tetrahedron 54, 14421–14436.

García-Granados, A., Martínez, A., Quirós, R., Extremera, A.L., 1999. Chemical-microbiological semisynthesis of enantio-Ambrox® derivatives. Tetrahedron 55, 8567–8578.

García-Granados, A., Melguizo, E., Parra, A., Pérez, F.L., Simeó, Y., Viseras, B., Arias, J.M., 2000a. Chemical semisynthesis and biotransformation with *Rhizopus nigricans* of several sesquiterpenes: obtention of new 1α- and 2α-hydroxyselinane derivatives. Tetrahedron 56, 6517–6526.

García-Granados, A., Melguizo, E., Parra, A., Simeó, Y., Viseras, B., Dobado, J.A., Molina, J., Arias, J.M., 2000b. Regioselective enzymatic acylations of polyhydroxylated eudesmanes: semisynthesis, theoretical calculations, and biotransformation of cyclic sulfites. J. Org. Chem. 65, 8214–8223.

Marles, R.J., Pazos-Sanou, L., Compadre, C.M., Pezzuto, J.M., Boszyk, E., Arnason, J.T., 1995. Sesquiterpene lactones revisited: recent developments in the assessment of biological activities and structure relationships. In: Arnason, J.T., Mata, R., Romeo, J.T., (Eds.), Phytochemistry of Medicinal Plants, Vol. 29. Plenum Press, New York, pp. 333–356.

Miyazawa, M., Honjo, Y., Kameoka, H., 1997. Biotransformation of the sesquiterpenoid β-selinene using the plant-pathogenic fungus *Glomerella cingulata* as a biocatalyst. Phytochemistry 44, 433–436.

Picman, A.K., 1986. Biological activities of sesquiterpene lactones. Biochem. Sys. Ecol 14, 255–281.

Robles, M., Aregullin, M., West, J., Rodríguez, E., 1995. Recent studies on the zoopharmacognosy, pharmacology and neurotoxicology of sesquiterpene lactones. Planta Med. 61, 199–203.

Rodríguez, E., Towers, G.H.N., Mitchell, J.C., 1976. Biological activities of sesquiterpene lactones. Phytochemistry 15, 1573–1580.

Schmidt, T.J., 1999. Toxic activities of sesquiterpene lactones: structural and biochemical aspects. Current Org. Chem. 3, 577–608.

Shimizu, N., Akita, H., Inamaya, S., Oishi, T., 1994. A formal synthesis of (–)-α-santonin from chiral α,β-epoxyeudesmanolide via enzyme-catalyzed hydrolysis. Chem. Pharm. Bull. 42, 1160–1162.

Sih, C.J., Rosazza, J.P.N., 1976. Microbial transformations in organic synthesis. In: Jones, J.B., Sih, C.J., Perlman, D. (Eds.), Applications of biochemical Systems in Organic Chemistry, Part 1. John Wiley and Sons Inc., New York, p. 69.

Socorro, O., 2001. Estudio de las Zahareñas, Rabos de Gato o Garranchuelos de la Provincia de Granada.