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The microbiological transformation of the diterpenes dehydroabietanol and teideadiol by *Mucor plumbeus*

Braulio M. Fraga[a,*], Melchor G. Hernández[a], Jose M. Artega[b], Sergio Suárez

[a]*Instituto de Productos Naturales y Agrobiología, CSIC, Avda. Astrofísico F. Sánchez 3, La Laguna, 38206-Tenerife, Canary Islands, Spain*
[b]*Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Tenerife, Spain*

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Abstract

The microbiological transformation of dehydroabietanol (18-hydroxy-dehydroabietane) by *Mucor plumbeus* led to 2α,18-dihydroxy-abieta-8,11,13,15-tetraene, 2α,15-dihydroxy-dehydroabietanol, 2α-hydroxy-15-methoxy-dehydroabietanol, 7β-hydroxy-2-oxo-dehydroabietanol, 15-hydroxy-2-oxo-dehydroabietanol and 15,16-dihydroxy-2-oxo-dehydroabietanol, whilst that of teideadiol (1α,18-dihydroxy-dehydroabietane) gave 2α-hydroxy-teideadiol, 7α-hydroxy-teideadiol and 7β-hydroxy-teideadiol. Thus, 2α- and 7β-hydroxylation occur in both biotransformations and the 15-hydroxylation is inhibited in the biotransformation of teideadiol by the presence of a 1α-alcohol.

Keywords: *Mucor plumbeus*; Biotransformations; Diterpenes; Dehydroabietanol; Teideadiol

1. Introduction

For the last 20 years we have been interested in the study of the microbiological transformation of diterpenes by fungi. One of the microorganisms employed has been *Mucor plumbeus,* which possesses a broad specificity in the substrate. The aim of these studies was the preparation of analogues of the bioactive diterpene forskolin, using as substrates manoyl oxide derivatives of the *normal* and *enantio* series (Fraga et al., 1998, 2001). Now we have continued these works, but using substrates with a different carbon skeleton, with a view to developing models to explain these diterpene microbiological hydroxylations by this fungus. On the other hand, some dehydroabietane diterpenes have been shown to possess antimicrobial activity against bacteria and fungi (Tapia et al., 1997; Gigante et al., 2002).

2. Results and discussion

The substrates used were dehydroabietanol (**1**) and teideadiol (**15**). The former has been obtained, mixed with its 4-epimer, from *Juniperus phoeniceae* (San Feliciano et al., 1992) and later from *Salvia pomifera*, and named pomiferin A (Ulubelen and Topcu, 1992), whilst the latter has been isolated from *Nepeta teydea* (Breton et al., 1970; Fraga et al., 1994). We have prepared **1** by lithium aluminum hydride reduction of dehydroabietic acid methyl ester (**3**) (Fraga et al., 1994).

The incubation of dehydroabietanol (**1**) with *M. plumbeus* gave the metabolites **4**, **7**–**10** and **12**. The main compound was **4**, which showed in its high resolution MS the molecular ion at m/z 318.2200 ($C_{20}H_{30}O_3$). The two new oxygens introduced in the molecule were due to two alcoholic groups, one secondary and one tertiary. Thus, in the ^{1}H NMR spectrum the geminal proton to the secondary hydroxyl resonates as a broad multiplet at δ 4.11 ($W_{1/2} = 23$ Hz), which is characteristic of a β-axial hydrogen at C-2. The tertiary alcohol was located at C-15, because in this spectrum the resonance of H-15 does not appear and the two methyls at C-15 resonate as a singlet at a low field, δ 1.55. The C-2 and C-15 bearing these new alcohols appear in the ^{13}C NMR spectrum at δ 65.5 and 72.3, respectively (Table 1). In consequence, the structure of this metabolite was determined to be 2α,15-dihydroxy-dehydroabietanol (**4**), which was confirmed by 2D NMR data analysis of this compound and those of their diacetate (**5**) and triacetate (**6**).

* Corresponding author. Tel.: +34-922-251728; fax: 34-922-260135.
E-mail address: bmfraga@ipna.csic.es (B.M. Fraga).

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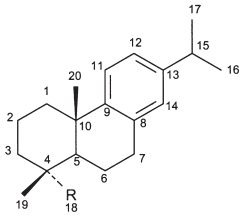
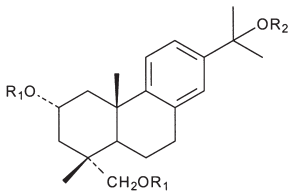
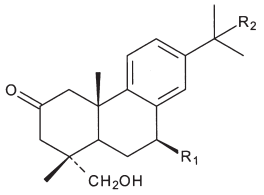


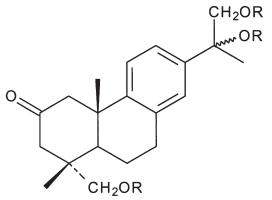
1 R = CH_2OH
2 R = CO_2H
3 R = CO_2Me



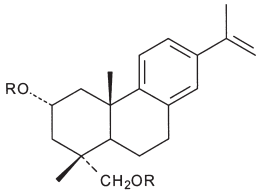
4 $R_1 = R_2 = H$
5 $R_1 = Ac$ $R_2 = H$
6 $R_1 = R_2 = Ac$
7 $R_1 = H$ $R_2 = CH_3$



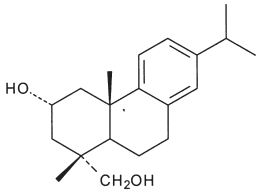
8 $R_1 = OH$ $R_2 = H$
9 $R_1 = H$ $R_2 = OH$



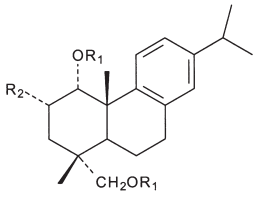
10 R = H
11 R = Ac



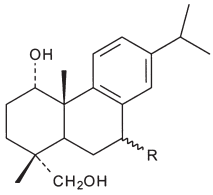
12 R = H
13 R = Ac



14



15 $R_1 = R_2 = H$
16 $R_1 = H$ $R_2 = OH$
17 $R_1 = D$ $R_2 = OD$



18 R = α-OH
19 R = β-OH

The second of the metabolites obtained in this feeding was **7** ($C_{21}H_{32}O_3$), which showed a structure very similar to that of **4**, the main difference between them being the presence of a tertiary methoxyl group, whose signals appear at δ 3.05 and δ 56.6 in the ^1H and ^{13}C NMR spectra, respectively. Therefore, we assigned the structure 2α-hydroxy-15-methoxy-dehydroabietanol (**7**) to this product, which was confirmed by study of its 2D NMR spectra (COSY, HSQC and HMBC).

To another compound obtained in the feeding of dehydroabietanol (**1**) the structure 7β-hydroxy-2-oxo-dehydroabietanol (**8**) was assigned on the basis of the following considerations: The molecular formula was $C_{20}H_{28}O_3$. The ^1H NMR spectrum showed the resonances of H-7 as a double doublet at δ 4.92 ($J = 10$ and 7 Hz), of the two H-1 at 2.49 (*d*, $J = 13.1$ Hz) and 2.95 (*dd*, $J = 13.1$ and 2 Hz), and of the two H-3 at δ 2.15 (*dd*, 14 and 2 Hz) and 2.76 (*d*, $J = 14$ Hz). A long distance *W*-coupling (2 Hz) was observed between H-1(β) and H-3(β). The carbon resonances of C-2 and C-7 appeared at δ 211.1 and 70.5, respectively. In the HMBC spectrum correlations were observed of H-1 with C-2, of C-5 with C-20, of H-3 with C-2, C-5, C-18 and C-19, and of H-7 with C-6, C-8 and C-9.

Table 1
^{13}C NMR data of compounds **1** and **4–8**

C	1	4	5	6	7	8
1	38.4	47.5	43.4	43.2	47.5	54.0
2	18.6	65.5	68.5	68.6	65.4	211.1
3	35.1	44.5	40.6	40.6	44.5	49.9
4	37.8	39.2	37.8	38.9	39.0	42.0
5	43.9	42.8	43.1	43.2	42.8	40.7
6	18.8	18.6	18.2	18.5	18.5	30.2
7	30.1	30.0	30.0	30.0	29.9	70.5
8	134.7	134.5	134.3	134.3	134.3	137.6
9	147.3	147.4	146.8	146.9	147.3	144.0
10	37.3	39.2	38.0	37.8	39.0	42.2
11	123.8	124.0	123.9	124.0	123.3	124.7
12	124.2	122.0	122.1	121.9	123.8	126.2
13	145.4	146.2	146.4	143.1	142.8	147.1
14	126.8	124.9	125.0	124.9	126.2	124.9
15	33.4	72.3	n.o.[a]	81.4	n.o	33.6
16	24.0	31.6	31.6	28.6	27.8	23.8
17	24.0	31.6	31.6	28.6	27.8	23.9
18	72.2	71.5	71.6	71.6	72.0	69.6
19	17.4	18.2	18.1	18.2	18.3	19.1
20	25.2	26.1	25.9	26.3	26.1	26.8

[a] n.o., Not observed, overlapped with the solvent.

To a metabolite, isomer of **8**, the structure 15-hydroxy-2-oxo-dehydroabietanol (**9**) was assigned considering that in its ^{13}C NMR spectrum the resonance of the carbonyl group at C-2 appears at δ 211.9. Its ^1H NMR spectrum showed a double doublet at δ 2.14 and a doublet at δ 2.75, corresponding to the two H-3, a doublet at δ 2.50 and a double doublet at δ 2.91 of the two H-1, and a singlet at δ 1.56, due to the methyls over C-15. The structure was confirmed by assignment of its ^1H and ^{13}C NMR spectra using 2D NMR techniques.

One of the minor compounds isolated from the feeding of **1** was **10**, which was obtained in the form of its triacetate **11,** by acetylation and chromatography of the fractions containing it. The molecular formula was $C_{26}H_{34}O_7$ determined by high resolution MS. Compound **11** was related with **9**, because rings A–C were identical, but now the 18-alcohol was in its acetate form (δ 3.73 and 3.98, each 1H doublet). The structural difference between both products was the presence of a functionalized isopropyl with an acetoxymethylene group, which appears in the ^1H NMR spectrum as a pair of doublets centred at δ 4.25 and 4.33. This chemi-

cal shift is characteristic of a dehydroabietane diterpene with a similar side chain (Yano et al., 1994). In consequence, the structure **10** (15,16-dihydroxy-2-oxo-dehydroabietanol) was assigned to the corresponding alcohol obtained in the fermentation. The stereochemistry at C-15 remains undetermined.

Finally, from this fermentation we isolated a very small amount of the metabolite **12**, which was impurified by a compound tentatively identified as **14**. The structure of the first was shown to be 2α, 18-dihydroxy-abieta-8,11,13,15-tetraene (**12**) on the basis of the following considerations: Its ^{1}H NMR spectrum showed signals of the two hydrogens at C-16 as two singlets at δ 5.00 and 5.30, whilst the methyl over C-15 appeared as a singlet at δ 2.11. The proton geminal to the 2α-alcohol appears as a broad multiplet at δ 4.12 ($W_{1/2}=24$ Hz) and the hydroxymethylene group at C-4 as a pair of doublets at δ 3.23 and 3.48. Dehydroabietane diterpenes with a 15-hydroxy or a 15,16-double bond have been isolated from the leaves of *Larix kaempferi* (Tanaka et al., 1997).

The incubation of teideadiol (**15**) with *M. plumbeus* led to the isolation of the metabolites **16**, **18** and **19**. The structure of **16**, which was obtained in good yield, was determined to be 2α-hydroxy-teideadiol. Its HRMS showed the molecular ion at *m*/*z* 318.2216 corresponding to the molecular formula $C_{20}H_{30}O_3$. The new oxygen introduced in the molecule during the incubation must form a part of a secondary hydroxyl group, because in its ^{1}H NMR spectrum there appears a new geminal proton at δ 4.21, resonating as a multiplet. When this spectrum was run in CD_3OD this signal was simplified appearing at δ 4.15 as a double doublet of doublets with coupling constants of 12.4, 4.7 and 2.5 Hz. This fact indicated that this geminal proton was also coupled with the hydrogen of the alcoholic group, which in deuterated methanol is interchanged for deuterium. This proton of the hydroxyl group resonates, in $CDCl_3$, at δ 2.23 as a doublet ($J=9.1$ Hz), which collapses to a singlet by irradiation of its geminal hydrogen at δ 4.21. The observed couplings only can be explained if this new alcohol is assigned to the C-2(α) position. This location was confirmed by double resonance experiments and 2D NMR data (COSY, NOESY, HMQC and HMBC), that also permitted unambiguous assignment of the ^{1}H and ^{13}C NMR spectra. In the MS spectrum the base peak was observed at *m*/*z* 186 ($C_{14}H_{18}$). This fragment (Fig. 1) may be formed by the cleavage of C-1, C-10 and C-4, C-5 bonds, indicating that the new hydroxyl group must be situated on ring A. Further proof of the composition of this fragment was obtained in the mass spectra of the deuterated compound **17** and of teideadiol (**15**), in which the base peak was also at *m*/*z* 186.

The structure of the second product obtained in this incubation was determined as 7α-hydroxy-teideadiol (**18**). The MS spectrum showed the molecular ion at

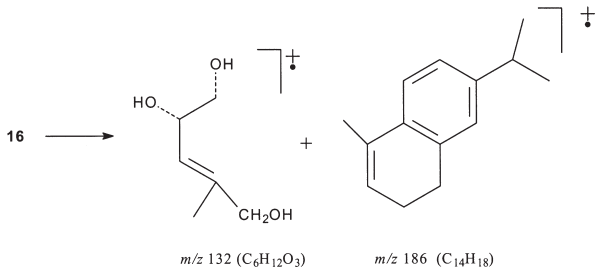


Fig. 1. A fragmentation of 2α-hydroxy-teideadiol (**16**).

318.2199 ($C_{20}H_{32}O_3$). The new hydroxyl group was located at C-7(α) considering the ^{1}H NMR spectrum, which showed the resonance of the geminal proton to the new alcohol as a broad singlet at δ 4.78. This relatively low value of resonance and the changes observed in the chemical shifts of the aromatic protons, with respect to the substrate, also indicated that the alcohol group must be allylic to the aromatic ring.

The third metabolite **19,** with a molecular formula $C_{20}H_{30}O_3$, was the 7β-epimer of **18**. The proton geminal to the 7β-hydroxyl resonates as a double doublet at δ 4.85 with coupling constants of 10.4 and 7.1 Hz, and H-14 is now shifted to lower field (δ 7.47), with respect to **18** (δ 7.24). The assignment of the ^{13}C NMR spectrum (Table 2) confirmed the C-7 position and the β-stereochemistry. Thus, C-5 now resonates at δ 35.5 in comparison with 30.5 in the spectrum of **18**, because in the latter there exists a syn-diaxial interaction between the 7α-OH and H-5.

Several conclusions can be obtained from these biotransformations:

Table 2
^{13}C NMR data of compounds **9**, **15**, **16**, **18** and **19**

C	**9**	**15**	**16**	**16**[a]	**18**	**19**
1	53.9	71.7	74.9	76.0	71.6	71.7
2	211.9	25.7	66.5	67.6	24.0	25.7
3	50.1	27.8	37.3	37.8	27.7	27.8
4	42.3	37.5	38.5	39.6	37.0	37.2
5	42.2	37.4	36.6	38.3	30.5	35.5
6	19.1	18.4	18.2	19.7	27.1	29.6
7	30.3	30.6	30.5	31.6	68.5	70.7
8	134.9	137.2	136.6	137.2	138.0	140.1
9	145.6	146.2	146.4	146.8	147.1	147.1
10	42.5	43.4	44.2	45.2	43.8	44.1
11	124.5	124.5	124.6	126.6	127.3	125.9
12	122.4	123.7	123.6	124.9	124.1	123.5
13	147.8	142.1	141.7	144.3	142.4	142.0
14	124.9	127.9	127.9	128.1	129.0	126.4
15	72.3	33.5	33.5	34.9	33.5	33.7
16	31.6	23.9	23.8	24.5	23.8	23.8
17	31.6	23.9	23.8	24.5	23.9	23.6
18	70.1	72.0	71.6	72.3	70.9	71.5
19	17.6	17.5	18.2	18.7	17.6	17.4
20	26.5	25.9	25.1	28.3	24.4	24.0

[a] Solvent: CD_3OD.

1. The C-2(α) and the C-7 positions are the main sites of the hydroxylations produced in the incubations of both substrates, dehydroabietanol (**1**) and teideadiol (**15**), with *M. plumbeus*. The 2α-hydroxylation was also the main reaction observed by us in the incubation of 18-hydroxymanoyl oxide by this fungus (Fraga et al., 1998).
2. The main difference between both biotransformations was the 15-hydroxylation of dehydroabietanol (**1**), which does not occur in teideadiol (**15**). This indicated that the 1α-alcohol in the latter inhibits the C-15 functionalization.
3. The 15- and 16-hydroxylations produced now in the incubation of **1** with *M. plumbeus* have also been observed in the biotransformation of dehydroabietic acid (**2**) by C*haetomium cochliodes* (Yano et al., 1994), *Fomes annosus* (Ekman and Sjöholm, 1979) and *Mortierella isabellina* (Kutney et al., 1981), which implies a similar functionalization carried out by enzyme(s) from four different genera of fungi. The last microorganism also produces the 2α-hydroxylation of dehydroabietic acid (**2**). However, the formation of a 2α-alcohol and its subsequent oxidation to a 2-oxo derivative, such as **8**–**10**, has only now been produced in the biotransformation of **1** with *M. plumbeus*.

3. Experimental

3.1. General

^{1}H NMR spectra were recorded in $CDCl_3$ solutions at 500.13 MHz with a Bruker AMX2-500 spectrometer. ^{13}C NMR spectra were run in $CDCl_3$ at 50.32 and 125.13 MHz with a Bruker AC-200 or a Bruker AMX2-500, respectively. Chemical shifts are given in ppm (δ). Mass spectra and HRMS were taken at 70 eV in a Micromass Autospec spectrometer. Dry column chromatographies were made on Si gel Merck 0.2–0.065 mm. A commercial sample of dehydroabietic acid (American Drugs, California, USA) has been used in this work.

3.2. Organism

The fungal strain, *Mucor plumbeus* CMI 116688, was a gift from Dr. J.R. Hanson, School of Chemistry, Physics and Environmental Sciences, University of Sussex, UK.

3.3. Incubation experiments

The fungus *Mucor plumbeus* was grown in shake culture at 25 °C, in conical flasks (250 ml), each containing 50 ml of a sterile medium comprising (per litre) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g) and trace elements solution (2 ml). The trace-elements solution contained (per 100 ml) $Co(NO_3)NO_2$ (0.01 g), $CuSO_4$, (0.015 mg), $ZnSO_4$ (0.16 g), $MnSO_4$ (0.01 g) and $(NH_4)_6Mo_7O_{24}$ (0.01 g). The substrate dissolved in EtOH was evenly distributed among flasks after 1 day of growth. After a further 6 days, the fermentation was harvested. The mycelium was filtered, and the culture filtrate was extracted with EtOAc. The extract was dried over Na_2SO_4 and the solvent evaporated to give a syrupy residue.

3.3.1. Incubation of dehydroabietanol (*1*)

The substrate (**1**, 250 mg) in 50 conical flasks was incubated as above. Chromatography of the residue on silica gel eluting with petrol–EtOAc mixtures gave: 2α,18-dihydroxy-abieta-8,11,13,15-tetraene (**12**) impurified by 2α-hydroxy-dehydroabietanol (**14**) (0.7 mg), 2α-hydroxy-15-methoxy-dehydro-abietanol (**7**, 4.2 mg), 15-hydroxy-2-oxo-dehydroabietanol (**9**, 1 mg), 7α-hydroxy-2-oxo-dehydroabietanol (**8**, 3.4 mg), 2α,15-dihydroxy-dehydroabietanol (**4**, 18 mg) and 15,16-dihydroxy-2-oxo-dehydroabietanol (**10**, 0.6 mg).

*3.3.1.1. 2α,15-Dihydroxy-dehydroabietanol (**4**).* Colourless prisms, m.p. 100–102 °C (MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.91 (3H, *s*, H-19), 1.23 (3H, *s*, H-20), 1.35 (1H, *t*, $J=11.8$ Hz, H-1), 1.42 (1H, *t*, $J=11.8$ Hz, H-3), 1.55 (6H, *s*, H-16 and H-17), 2.62 (1H, *ddd*, $J=12.1$, 3.9 and 2.1 Hz, H-1), 2.91 (2H, *m*, H-7), 3.22 and 3.48 (each 1H, *d*, $J=11$ Hz, H-18), 4.11 (1H, *br m*, $W_{1/2}=23$ Hz, H-2), 7.17 (1H, *br s*, H-14), 7.21 (2H, *s*, H-11 and H-12); ^{1}H NMR (500 MHz, CD_3OD): δ 0.89 (3H, *s*, H-19), 1.22 (3H, *s*, H-20), 1.24 (1H, *m*, H-1), 1.48 (6H, *s*, H-16 and H-17), 1.49 (1H, *m*, H-3), 1.66 and 1.83 (each 1H, *m*, H-6), 2.59 (1H, *ddd*, $J=12.1$, 3.9 and 2.0 Hz, H-1), 2.90 (2H, *m*, H-7), 3.11 and 3.43 (each 1H, *d*, $J=11$ Hz, H-18), 4.05 (1H, *br m*, $W_{1/2}=23$ Hz, H-2), 7.12 (1H, *d*, $J=2.1$ Hz, H-14), 7.17 (1H, *dd*, $J=8.2$ and 2.1 Hz, H-12), 7.19 (1H, *d*, $J=8.2$ Hz, H-11); $[M]^+$ at *m*/*z* 318.2200, $C_{20}H_{30}O_3$ requires 318.2194. *Diacetate* (**5**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01 (3H, *s*, H-19), 1.28 (3H, *s*, H-20), 1.46 (1H, *t*, $J=11.7$, H-1β), 1.49 (1H, *t*, $J=12.2$ Hz, H-3β), 1.54 (6H, *s*, H-16 and H-17), 1.67 (1H, *br d*, $J=10.3$ Hz, H-5), 1.74 and 1.78 (each 1H, *m*, H-6), 1.83 (1H, *br d*, H-3α), 2.04 and 2.07 (each 3H, *s*,–OAc), 2.63 (1H, *br d*, $J=11.7$ and 1.9 Hz, H-1β), 2.85 (1H, *m*, H-7α), 2.96 (1H, *dd*, $J=17$ and 6.1 Hz, H-7β), 3.71 and 3.99 (each 1H, *d*, $J=11$ Hz, H-18), 5.22 (1H, *m*, $W_{1/2}=26$ Hz, H-2α), 7.19 (1H, *br s*, H-14), 7.21 (1H, *d*, $J=8.4$ Hz, H-11), 7.25 (1H, *br d*, $J=8.4$ Hz, H-12); EIMS *m*/*z* (rel. int.): 402 $[M]^+$ (10), 384 (27), 342 (5), 327 (42), 324 (13), 309 (25), 267 (100), 249 (89), 239 (7), 209 (20), 207 (11), 173 (44), 157 (18), 134 (36); $[M]^+$ at *m*/*z* 402.2397. $C_{24}H_{34}O_5$ requires 402.2406. *Triacetate* (**6**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (3H, *s*, H-19), 1.28 (3H, *s*, H-20), 1.45 (1H, *t*, $J=11.7$ Hz, H-1α), 1.51

(1H, *t*, *J* = 12.3 Hz, H-3α), 1.67 (1H, *br d*, *J* = 11.6, H-5), 1.70 (1H, *m*, H-6), 1.73 (6H, *s*, H-16 and H-17), 1.87 (2H, *m*, H-3β and H-6), 2.03, 2.06 and 2.07 (each 3H, *s*,–OAc), 2.60 (1H, *dd*, *J* = 11.7 and 1.9 Hz, H-1β), 2.83 (1H, *m*, H-7α), 2.92 (1H, *dd*, *J* = 16.7 and 6.2 Hz, H-7β), 3.72 and 3.99 (each 1H, *d*, *J* = 11.2 Hz, H-1β), 5.23 (1H, *m*, $W_{1/2}$ = 23 Hz, H-2α), 7.00 (1H, *br s*, H-14), 7.09 (1H, *br d*, *J* = 8.4 Hz, H-12), 7.17 (1H, *d*, *J* = 8.4 Hz, H-11); EIMS *m*/*z* (rel. int.): 318 $[M]^+$ (26), 303 (78), 300 (46), 285 (27), 267 (96), 249 (100), 237 (24), 209 (35), 181 (36) 171 (52). $[M]^+$ at *m*/*z* 444.2493, $C_{26}H_{36}O_6$ requires 444.2511.

*3.3.1.2. 2α-Hydroxy-15-methoxy-dehydroabietanol (**7**).* A gum; ^{1}H NMR (500 MHz, CD_3OD): δ 0.93 (3H, *s*, H-19), 1.26 (3H, *s*, H-20), 1.36 (1H, *t*, *J* = 11.8 Hz, H-1α), 1.44 (1H, *t*, *J* = 11.8 Hz, H-3α), 1.49 (6H, *s*, H-16 and H-17), 1.72 (1H, *m*, H-6), 1.77 (1H; *ddd*, *J* = 12, 3.8 and 1.8 Hz, H-3β), 1.81 (1H, *m*, H-6), 2.64 (1H, *ddd*, *J* = 11.8, 3.9 and 1.7 Hz, H-1β), 2.91 (2H, *m*, H-7), 3.05 (3H, *s*, –OMe), 3.25 and 3.50 (each 1H, *d*, *J* = 11 Hz, H-18), 4.14 (1H, *m*, $W_{1/2}$ = 23 Hz, H-2α), 7.06 (1H, *d*, *J* = 1.7 Hz, H-14), 7.15 (1H, *dd*, *J* = 8.3 and 1.7 Hz, H-12), 7.22 (1H, *d*, *J* = 8.3 Hz, H-11); EIMS *m*/*z* (rel. int.): 332 $[M]^+$ (3), 317 (100), 303 (8), 301 (5), 285 (3), 267 (4), 203 (3), 201 (3), 183 (3), 171 (4); $[M]^+$ at *m*/*z* 332.2336. $C_{21}H_{32}O_3$ requires 332.2351.

*3.3.1.3. 7β-Hydroxy-2-oxo-dehydroabietanol (**8**).* A gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-19), 1.24 and 1.25 (each 3H, *s*, H-16 and H-17), 1.32 (3H, *s*, H-20), 1.73 (1H, *dt*, *J* = 12.6 and 10.5, H-6β), 2.15 (1H, *dd*, *J* = 14 and 2 Hz, H-3β), 2.28 (1H, *dd*, *J* = 12.2 and 7.0 Hz, H-6α), 2.39 (1H, *dd*, *J* = 12.6 and 1.3 Hz, H-5), 2.49 (1H, *d*, *J* = 13.1 Hz, H-1α), 2.76 (1H, *d*, *J* = 14 Hz, H-3α), 2.89 (1H, sept, *J* = 7 Hz, H-15), 2.95 (1H, *dd*, *J* = 13.1 and 2 Hz, H-1β), 3.16 and 3.66 (each 1H, *d*, *J* = 10.9 Hz, H-18), 4.92 (1H, *dd*, *J* = 10.0 and 7.0 Hz, H-7), 7.07 (1H, *d*, *J* = 8.3, H-11), 7.13 (1H, *dd*, *J* = 8.3 and 2.0 Hz, H-12), 7.42 (1H, *d*, *J* = 2 Hz, H-14); EIMS *m*/*z* (rel. int.): 316 $[M]^+$ (53), 301 (36), 283 (5), 265 (12), 249 (7), 237 (28), 223 (8), 197 (23), 195 (21), 193 (12), 183 (33), 181 (20), 171 (10), 169 (10), 162 (100), 155 (14), 149 (12), 143 (24), 141 (16), 129 (12); $[M]^+$ at *m*/*z* 316.2081. $C_{20}H_{28}O_3$ requires 316.2038.

*3.3.1.4. 15-Hydroxy-2-oxo-dehydroabietanol (**9**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (3H, *s*, H-19), 1.25 (3H, *s*, H-20), 1.56 (6H, *s*, H-16 and H-17), 2.14 (1H, *dd*, *J* = 13.7 and 2 Hz, H-3β), 2.31 (1H, *dd*, *J* = 12.2 and 2.2 Hz, H-5), 2.50 (1H, *d*, *J* = 13 Hz, H-1α), 2.75 (1H, *d*, *J* = 13.7 Hz, H-3α), 2.91 (1H, *dd*, *J* = 13 and 2 Hz, H-1β), 2.94 (1H, *m*, H-7), 3.15 and 3.64 (each 1H, *d*, *J* = 11 Hz, H-18), 7.12 (1H, *d*, 8.6 Hz, H-11), 7.21 (1H, *br s*, H-14), 7.24 (1H, *br d*, *J* = 8 Hz, H-12) ; EIMS *m*/*z* (rel. int.): 316 $[M]^+$ (15), 301 (70), 283 (86), 281 (11), 280 (47), 265 (98), 251 (49), 239 (42), 237 (100), 223 (77), 209 (20), 197 (48), 183 (80), 181 (62), 165 (58), 157 (30), 141 (62), 129 (61), 115 (60); $[M]^+$ at *m*/*z* 316.2055. $C_{20}H_{28}O_3$ requires 316.2038.

*3.3.1.5. 15,16-Dihydroxy-2-oxo-dehydroabietanol (**10**).* Obtained in the form of its triacetate by acetylation, with Ac_2O–pyridine (2:1) at reflux for 6 h, and chromatography, of the fractions containing it. *Triacetate* (**11**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (3H, *s*, H-19), 1.26 (3H, *s*, H-20), 1.55 (3H, *s*, H-17), 2.07 (9H, *s*, 3-OAc), 2.12 (1H, *dd*, *J* = 14 and 2 Hz, H-3β), 2.26 (1H, *dd*, *J* = 12 and 2 Hz, H-5), 2.53 (1H, *d*, *J* = 13.5 Hz, H-1α), 2.65 (1H, *d*, *J* = 14 Hz, H-3α), 2.99 (1H, *dd*, *J* = 13.5 Hz and 2 Hz, H-1β), 3.73 and 3.98 (each 1H, *d*, *J* = 11. 5 Hz, H-18), 4.25 and 4.33 (each 1H, *d*, *J* = 11.6 Hz, H-16), 7.00 (3H, *s*, H-14), 7.08 (2H, *s*, H-11 and H-12); EIMS *m*/*z* (rel. int.): 458 $[M]^+$ (3), 398 (33), 370 (42), 356 (57), 343 (35), 323 (44), 296 (52), 281 (75), 267 (38), 249 (60), 197 (46), 179 (62), 165 (100), 155 (55), 152 (55), 141 (63), 128 (73), 115 (81); $[M]^+$ at *m*/*z* 458.2339. $C_{26}H_{34}O_7$ requires 358.2304.

*3.3.1.6. 2α,18-Dihydroxy-abieta-8,11,13,15-tetraene (**12**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-19), 1.25 (3H, *s*, H-20), 2.11 (3H, *s*, H-17), 3.23 and 3.48 (each 1H, *d*, *J* = 11 Hz, H-18), 4.12 (1H, *m*, $W_{1/2}$ = 24 Hz, H-2α), 5.00 and 5.30 (1H, *br s*, H-16), 7.16 (1H, *br s*, H-14), 7.21 (1H, *d*, *J* = 8.1 Hz, H-11), 7.26 (1H, *br d*, *J* = 8 Hz, H-12); $[M]^+$ at *m*/*z* 300.2134. $C_{20}H_{28}O_2$ requires 300.2089. *Acetate* (**13**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.04 (3H, *s*, H-19), 1.25 (3H, *s*, H-20), 2.03 and 2.07 (each 3H, *s*, 2-OAc), 2.12 (3H, *s*, H-17), 2.63 (1H, *ddd*, *J* = 12, 3.8 and 2.2 Hz, H-1β), 2.85 (1H, *m*, H-7α), 2.96 (1H, br *dd*, *J* = 16 and 5 Hz, H-7β), 3.72 and 4.00 (each 1H, *d*, *J* = 11 Hz, H-18), 5.04 and 5.33 (each 1H, *s*, H-16), 7.16 (1H, *br s*, *J* = 8 Hz, H-14), 7.20 (1H, *d*, *J* = 8 Hz, H-11), 7.26 (1H, *br d*, *J* = 8, H-12); $[M]^+$ at *m*/*z* 384.2351. $C_{24}H_{32}O_4$ requires 384.2301.

*3.3.2. Incubation of teideadiol (**15**)*

The substrate (**15**, 290 mg) in 60 conical flasks was incubated as above. Chromatography of the extract on silica gel eluting with petrol–EtOAc mixtures gave 7β-hydroxy-teideadiol (**19**, 2 mg), 2α-hydroxy-teideadiol (**16**, 33.6 mg) and 7α-hydroxy-teideadiol (**18**, 3.5 mg).

*3.3.2.1. 2α-Hydroxy-teideadiol (**16**).* Colourless prisms, m.p. 214–216 (petrol-EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-19), 1.22 and 1.23 (each 3H, 5, H-16 and H-17), 1.26 (3H, *s*, H-20), 1.50 (1H, *dd*, *J* = 12.4 and 4.9, H-3), 1.89 (1H, *t*, *J* = 12.4 Hz, H-3), 2.10 (lH, *dd*, *J* = 2 and 12.4, H-5), 2.23 (1H, *d*, *J* = 9.1 Hz,-OH), 2.82 (1H, *sept*., H-5), 2.88 (1H, *m*, H-7), 3.26 and 3.51 (each 1H, *d*, *J* = 11 Hz, H-18), 4.21 (1H, *m*, H-2), 4.40 (1H, *s*, H-1), 6.94 (1H, *d*, *J* = 1.9 Hz, H-14), 7.0 (1H, *dd*, *J* = 8 and 1.9 Hz, H-12), 7.20 (1H, *d*, *J* = 8 Hz,

11-H); ^{1}H NMR (500 MHz, CH_3OD): δ 0.90 (3H, *s*, H-19), 1.67 (3H, *s*, H-20), 1.81 and 1.90 (each 3H, *s*, H-16 and H-17), 1.36 (lH, *dd*, $J=12.4$ and 4.6, H-3), 1.70 and 1.77 (each 1H, *m*, H-6), 1.89 (1H, *t*, $J=12.5$ Hz, H-3), 2.06 (1H, *dd*, $J=12.4$ and 2.1 Hz, H-5), 2.76 (1H, *m*, H-15), 2.84 (2H, *m*, H-7), 3.18 and 3.40 (each 1H, *d*, $J=11$ Hz, H-18), 4.15 (1H, *ddd*, $J=12.4$, 4.7 and 2.5 Hz, H-2), 4.28 (1H, *d*, $J=2.5$, H-1), 6.84 (1H, *d*, $J=1.9$ Hz, H-14), 6.97 (1H, *dd*, $J=8.1$ and 1.9 Hz, H-12), 7.20 (1H, *d*, $J=8.1$ Hz); EIMS *m*/*z* (rel. int.): 318 $[M]^+$ (64), 303 (12), 300 (29), 285 (26), 282 (38), 269 (55), 267 (60), 264 (77), 251 (54), 249 (92), 239 (71), 237 (50), 227 (30), 209 (61), 195 (72), 186 (100), 181 (34), 179 (36), 171 (39), 165 (40), 155 (30), 143 (47). $[M]^+$ at *m*/*z* 318.2216. $C_{20}H_{30}O_3$ requires 318.2194.

*3.3.2.2. 7α-Hydroxy-teideadiol (**18**)*. A gum; ^{1}H NMR (500 MHz): δ 0.85 (3H, *s*, H-19), 1.20 (3H, *s,* H-20), 1.25 and 1.26 (each 1H, *s*, H-16 and H-17), 2.88 (1H, *sept*., H-15), 3.12 and 3.61 (each 1H, *s*, $J=11.4$ Hz, H-18), 4.34 (1H, *br s*, H-1), 4.78 (1H, *br s*, H-7), 7.21 (2H, *br s*, H-11 and H-12), 7.24 (1H, *br s*, H-14); EIMS *m*/*z* (rel. int.): $[M]^+$ 318 (7), 307 (12), 300 (6), 282 (12), 267 (21), 264 (16), 251 (100), 237 (11), 209 (39), 193 (20), 184 (38), 264 (16), 167 (24), 165 (24), 149 (52), 143 (45); $[M]^+$ at *m*/*z* 318.2199. $C_{20}H_{30}O_3$ requires 318.2195.

*3.3.2.3. 7β-Hydroxy-teideadiol (**19**)*. A gum; ^{1}H NMR (500 MHz): δ 0.89 (3H, *s*, H-19), 1.24 and 1.25 (each 1H, *s*, H-16 and H-17), 1.31 (3H, *s*, H-20), 2.90 (1H, *sept*., H-15), 3.26 and 3.52 (each 1H, *d*, $J=10.8$, H-18), 4.35 (1H, *br s*, H-1), 4.85 (1H, *dd*, $J=10.4$ and 7.1 Hz, H-7), 7.15 (1H, *dd*, $J=8$ and 1.9 Hz, H-12), 7.22 (1H, *d*, $J=8$ Hz, H-11), 7.47 (1H, *d*, $J=1.9$ Hz, H-14); EIMS *m*/*z* (rel. int.): $[M]^+$ 318 (3), 300 (3), 282 (8), 267 (13), 251 (100), 236 (6), 221 (7), 209 (24), 193 (12), 184 (19), 167 (14), 155 (6), 143 (19); $[M]^+$ at *m*/*z* 318.2200. $C_{20}H_{30}O_3$ requires 318.2195.

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