J. Chem. Research (S), 1999, 396–397†

Microbial Transformation of (+)-10β,14-Dihydroxy-*allo*-aromadendrane and (−)-*allo*-Aromadendrone†

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The biotransformation of (+)-10β,14-dihydroxy-*allo*-aromadendrane **1** and (−)-*allo*-aromadendrone **2** by *Beauvaria densa* CMC 3240, *Beauvaria bassiana* ATCC 7159, *Curvularia lunata* 2380 and *Rhizopus* sp. (from grapes) is investigated.

Sesquiterpenes are very widespread constituents of essential oils. Some of them are of considerable industrial value in the flavour and perfumery industries as well as in the manufacture of pharmaceutical products.[1] Total synthesis of selected analogues of these compounds is often quite difficult, so alternative ways to produce them must be sought, for example by microbiological derivation of natural materials. The biotransformation may lead to substitution at chemically inaccessible sites without perturbing the underlying carbon skeleton of the substrate.[2]

The sesquiterpenes **1** and **2** were used as starting substrates. Compound **1** is a natural product isolated from *Pulicaria paludosa*[3] and *Duguetia glabriuscula*[4] and compound **2** is readily prepared from **1** by treatment with $NaIO_4$.[5] (Fig. 1).

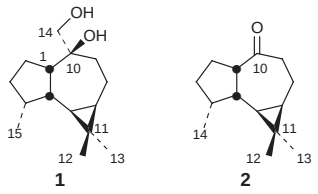

Fig. 1 Substrates for biotransformation

Beauvaria densa CMC 3240, *Beauvaria bassiana* ATCC 7159, and *Curvularia lunata* NRRL 2380 regioselectively oxidised **1** and **2** at the geminal methyl groups on the cyclopropane ring producing the triols **3** and **4** and the hydroxyketone **5**. The combined yields of compounds **3** and **4** were notably high (45–56%) for a biohydroxylation process. Furthermore, *C. lunata* converted **2** into the diol **6** as a result of hydroxylation at C(13) and concurrent reduction of the ketone carbonyl group at C(10). *Rhizopus* sp. (isolated from grapes) oxidised **2** at C(1) to form **7**, (See Fig. 2 and Table 1).

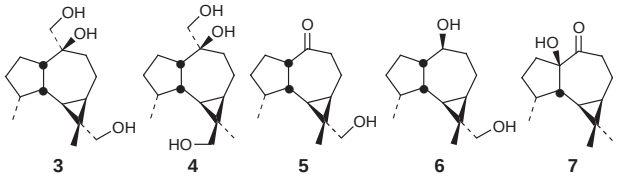

Fig. 2 Products from biotransformation

Table 1 Biotransformation of (+)-10β,14-dihydroxy-*allo*-aromadendrane **1** and (−)-*allo*-aromadendrone **2**

Microorganism	Substrate	Reaction time/days	Product	Yield(%)
B. densa	**1**	8–12	**3**	4.6
CMC 3240			**4**	38.0
B. bassiana	**1**	7–8	**3**	28.0
ATCC 7159			**4**	28.0
C. lunata	**1**	7–8	**3**	24.0
NRRL 2380			**4**	27.0
B. densa	**2**	8	**5**	8.5
CMC 3240				
B. bassiana	**2**	8	**5**	16.2
ATCC 7159				
C. lunata	**2**	8–10	**5**	11.7
NRRL 2380			**6**	12.1
Rhizopus sp.	**2**	5–8	**7**	17.3

The structure of compound **3** was identified as 10β,13,14-trihydroxy-*allo*-aromadendrane by comparing ^1H and ^{13}C NMR spectral data with those in the literature.[7] The ^1H NMR spectrum of **4** displayed two signals at δ 3.6 and 3.5 due to the primary alcohol group. The methyl signal at $CH_3(12)$ had disappeared. The ^{13}C NMR spectrum of **4** had a signal at δ 64.2 for C(12), a shift of 48.1 ppm. The signal for C(13) had moved to δ 19.4 representing an upfield shift of 9.7 ppm. From these data the metabolite **4** was identified as 10β,12,14-trihydroxy-*allo*-aromadendrane.

The metabolite **5** was identified as 13-hydroxy-*allo*-aromadendrone by comparing ^1H and ^{13}C NMR spectral data with those obtained for ketone **2**. The ^{13}C NMR spectrum of **5** showed that C(13) had moved to δ 73.7 with a downfield shift of 45.6 ppm. The signal for C(12) was at δ 11.8 with an upfield shift of 4.4 ppm. The diol **6** was the major product from the incubation of **2** with *C. lunata*. The stereochemistry of the hydroxylation as well as the orientation of the hydroxy group formed by reduction of ketone carbonyl was assigned by a NOE experiments. Irradiation at H-6 resulted in an enhancement of H(7), H(10) and H(13). Additionally, irradiation at δ 3.93 [H(10)] resulted in enhancement of H(6) and H(7). The metabolite was characterised as 10β,13-dihydroxy-*allo*-aromadendrane. From the incubation of **2** with *Rhizopus* sp. was isolated only 1-hydroxy-*allo*-aromadendrone **7** and the structure was determined by comparing ^1H and ^{13}C NMR spectral data with those in the literature.[8]

As previously reported,[2] in all the produced metabolites the cyclopropane ring remained intact, hydroxylation taking place at an adjacent position. Although compound **3** is known, it was previously reported as a metabolite of the incubation of *allo*-aromadendrene with *Glomerella cingulata*.[7] The hydroxylation at C(12), which gave the new triol **4**, is not frequently observed in the microbial oxidation of sesquiterpenes. 13-Hydroxy-*allo*-aromadendrone **5** is a novel compound. Isolation of metabolite

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† This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1999, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

6 was particularly intriguing arising from concomitant oxidation and reduction in the same reaction vessel. This presumably results from compartmentalisation of the enzymes responsible within the cell or a switch between oxidative and reductive metabolism during the fermentation. Compound **7** has been obtained by microbiological methods for the first time. Low conversions are normally attained for these biotransformations; in most reactions reported here, 40% of the starting materials were recovered, so that, yields of products based on starting materials consumed were generally in the range 20–57%.

Experimental

^{1}H and ^{13}C NMR spectra were determined at 400 or 300 and 100 or 75 MHz respectively. Chemical shifts were measured in ppm downfield of TMS and coupling constants (J) in Hz (see Tables 2-4). IR spectra were determined in CH_2CI_2. Mass spectra were recorded on a TRIO1000 instrument. TLC was performed on Merck 60 F_{254} precoated silica plates and spots were detected by spraying with a solution of 1.5% $NH_4Mo_2O_2$, 1% $Ce^{IV}SO_4$ and 10% H_2SO_4 followed by charring. Flash chromatography was performed with Si-60 (Merck, 40–63 μm). The microorganisms were precultivated at 28 °C and 120 rpm in 100 mL conical flasks containing 100 mL of the following medium: 5% malt extract and distilled water. After 96 h, 100 mg of substrate dissolved in 0.5 mL EtOH were added to the cultures. Every day, starting 24 h after the substrate addition, samples were taken and analysed. Culture medium and mycelia were both separated by filtration and extracted (×3) with EtOAc. The solvent was evaporated and the crude extract separated on Si-60 columns with the appropriate light petroleum–EtOAc gradient.

10*β*,14-*Dihydroxy*-allo-*aromadendrane* **1**. $[\alpha]_D^{20} + 4.53$ ($CHCl_3$; *c* 1). $\nu_{max}(CH_2Cl_2$ cast)/cm^{-1} 3292, 2950, 2867, 1453, 1029. EIMS *m/z* (rel. int.): $[M - H_2O]^+$ 220.15 (15), 207.15 (21), 203.15 (40), 189.05 (60), 81.95 (14), 40.90 (100).

Allo-*Aromadendrone* **2**. $[\alpha]_D^{20} - 8.32$ ($CHCl_3$; *c* 0.8). $\nu(CH_2Cl_2$ cast)/cm^{-1} 2961, 2874, 1733, 1695, 1459, 1000. EIMS *m/z* (rel. int.): $[M]^+$ 206 (15), 163 (12), 83 (38), 69 (100).

10*β*,13,14-*Trihydroxy*-allo-*aromadendrane* **3**. $\nu_{max}(CH_2Cl_2$ cast)/cm^{-1} 3335, 2957, 2871, 1460, 1020. CIMS *m/z*. Found: $[M - H_2O + NH_4]^+$, 254.21210. $C_{15}H_{28}NO_2$ requires 254.21200.

10*β*,12,14-*Trihydroxy*-allo-*aromadendrane* **4**. $\nu_{max}(CH_2Cl_2$ cast)/cm^{-1} 3399, 2958, 2874, 1457, 1020. CIMS *m/z*. Found: $[M + NH_4]^+$, 272.22262. $C_{15}H_{30}NO_3$ requires 272.22257.

13-*Hydroxy*-allo-*aromadendrone* **5**. ν_{max} (CH_2Cl_2 cast)/cm^{-1} 3615, 2959, 1696, 1458, 1015. EIMS *m/z*. Found: $[M]^+$, 222.16180. $C_{14}H_{22}O_2$ requires 222.16199.

10*β*,13-*Dihydroxy*-allo-*aromadendrane* **6**. $\nu_{max}(CH_2Cl_2$ cast)/cm^{-1} 3615, 3460, 3007, 2957, 2874, 1466, 1011. CIMS *m/z*. Found: $[M + NH_4 - H_2O]^+$, 224.20181. $C_{14}H_{26}NO$ requires 224.20144.

1-*Hydroxy*-allo-aromadendrone **7**. $\nu_{max}(CH_2Cl_2$ cast)/cm^{-1} 3597, 3439, 2970, 2858, 1703, 1676, 1462, 1261, 1096, 1027. EIMS *m/z*. Found: $[M]^+$, 222.16202. $C_{14}H_{22}O_2$ requires 222.16199.

Table 2 ^{13}C NMR data of compounds **1**–**7** (100 MHz, $CDCl_3$, **1** 375 MHz, **4**, in CD_3OD)[a]

C	1	2	3	4	5	6	7
1	53.6	54.6	53.5	64.2	55.9	51.5	90.4
2	32.1	31.6	31.7[b]	30.5[b]	32.5	30.1[b]	36.2[b]
3	24.6	25.2	24.5[b]	24.5[b]	25.2	32.0[b]	30.5[b]
4	40.1	38.8	38.5	39.6	39.7	39.2	36.1
5	38.2	40.1	38.9	41.0	40.1	39.7	49.1
6	22.8	25.0	25.7[c]	25.0[c]	21.9	20.7[c]	23.1[c]
7	29.5	25.9	19.4[c]	25.6[c]	22.8	21.1[c]	27.2[c]
8	18.3	18.8	17.9[b]	25.5[b]	19.3	18.9[b]	19.2[b]
9	29.1	43.6	29.1[b]	33.1[b]	44.7	35.1[b]	40.5[b]
10	76.4	206.9	76.1	77.4	209.9	74.8	212.4
11	19.1	17.5	26.1	23.9	25.4	25.4	19.0
12	16.1[b]	15.3	11.7	64.2	11.8	11.4	15.5
13	29.1	28.1	73.6	19.4	73.7	74.2	28.1
14	70.8	15.1	70.7	71.6	15.9	16.3	15.2
15	16.2	–	16.3	17.0	–	–	–

[a] The data were consistent with those reported in the literature,[3–6] however, in this work a detailed assignment was accomplished. [b,c]The assignments for these signals within the same column may be interchanged.

Table 3 ^{1}H NMR data of compounds **1**–**7** (400 MHz, $CDCl_3$, **1** and **3**, 300 MHZ, **4**, in CD_3OD)[a]

H	1	2	3	4	5	6	7
1	1.90m	3.09m	1.90m	1.90m	3.20m	2.00m	—
2	1.50m[b]	1.27m	1.50m[b]	1.30m[b]	1.30m	1.30m[b]	1.35m[b]
	1.70m[b]	1.60m	1.70m[b]	1.60m[b]	1.60m	1.50m[b]	2.50m[b]
3	1.30m	1.43m	1.30m[b]	1.30m[b]	1.50m	1.70m[b]	1.35m[b]
	1.70m[b]	2.36m	1.70m[b]	1.60m[b]	2.40m	2,00m[b]	2.50m[b]
4	2.00m	1.98m	2.00m	2.00m	2.01m	1.90m	1.90m
5	1.80m	2.25m	1.80m	1.70m	2.40m	1.70m	1.75dd (10.5, 6.5)
6	0.12dd (9.0)	0.36dd (9.1, 11.1)	0.32dd (9.5)	0.28dd (9.1)	0.55dd (9.5, 11.0)	0.41dd (9.2, 11.2)	0.21dd (9.3, 10.6)
7	0.66	0.64ddd (4.7, 9.1, 13.7)	0.83ddd (6.0, 9.5, 10.5)	0.77m	0.80ddd (4.9, 9.5, 11.8)	0.72ddd (5.2, 9.2, 12.0)	0.72ddd (5.2, 9.3, 12.0)
8	1.50m[b]	1.63m	1.50m[b]	1.45m[b]	1.65m	1.50m[b]	1.35m[b]
	1.70m[b]	1.83m	1.70m[b]	1.65m[b]	1.80m	1.70m[b]	1.90m
9	1.50m[b]	2.44m	1.50m[b]	1.45m[b]	2.50m	1.40m[b]	3.01ddd
	1.70m[b]	2.53m	1.70m[b]	1.65m[b]	2.70m	1.60m[b]	(3.7, 5.1, 13.6) 2,48m[b]
10	—	—	—	—	—	3.90	—
12	1.01s	1.04s	1.15s	3.50d (12.0) 3.60d (12.0)	1.20s	1.09s	0.99s
13	1.03s	1.00s	3.26d (11.0) 3.47d (11.0)	1.10s	3.20d (11.0) 3.40d (11.0)	3.24d (10.8) 3.42d (10.8)	0.96s
14	3.41d (10.9) 3.28d (10.9)	0.94d(6.8)	3.29d (10.5) 3.42d 910.5)	3.20d (11.0) 3.30d (11.0)	0.98d(6.9)	0.96d(6.8)	0.94d(6.6)
15	0.93d(6.6)	—	0.95d(7.0)	0.96d(5.5)	—	—	—
OH							2.95s br

[a]The data were consistent with those reported in the literature,[3–8] however, in this work a detailed assignment was accomplished. [b]The assignments for these signals within the same column may be interchanged.

Table 4 NOE observed for compounds **2** and **6**

	2	6
H	Enhanced protons	Enhanced protons
6	7, 14, 13	7, 10, 13
10	6, 14, 13	6, 7

We are grateful to CAPES-Brazil for financial support to Dênis P. de Lima. We are also in debt to Dr João M. de Siqueira (for collecting *D. glabriuscula* and providing information about bioactivity of the substrates) and, Dr Mike Paylor, for growing *Rhizopus* sp. We also thank Lucinéia Vizzotto for samples of the substrates over the duration of this work, and Dr Paul A. Leonard for help on the use of various NMR techniques.

Received, 15th February 1999; Accepted, 16th March 1999
Paper E/9/01226E

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