Hydroxylation of Aromadendrane Derivatives by *Mucor plumbeus*†

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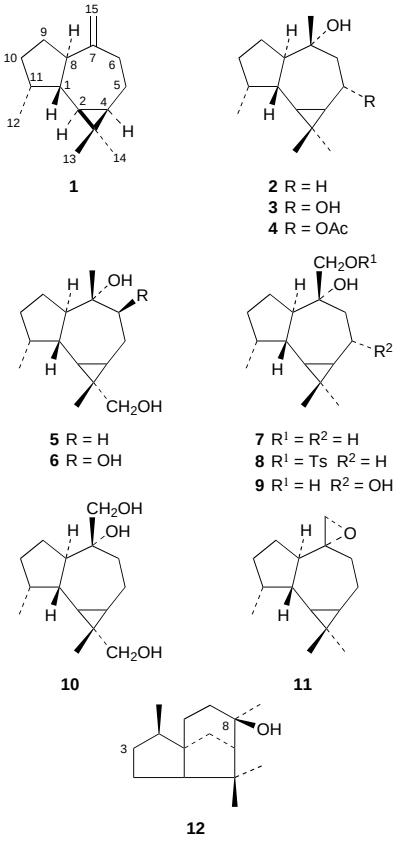
Both 7α-hydroxy- and 7α,15-dihydroxy-aromadendrane are efficiently hydroxylated by *Mucor plumbeus* at C-14 with retention of the adjacent cyclopropane ring.

The microbiological hydroxylation of sesquiterpenoids is a useful adjunct to chemical transformations.[1,2] Hydroxylated aromadendranes occur widely[3] and aromadendr-7(15)-ene (**1**) itself is readily available from Eucalyptus oils.[4] Since both *cis*- and *trans*-fused aromadendranes occur in closely related species, there is a need to prepare unambiguously hydroxylated aromadendranes for correlation purposes. Biotransformation may lead to substitution at chemically inaccessible sites without perturbing the underlying carbon skeleton of the substrate. The hydroxylation of aromadendranes by *Diplodia gossypina*,[5] *Cephalosporium aphidicola*[6] and *Glomerella cingulata*[7] has been reported previously and we now report the action of *Mucor plumbeus*, an organism that has been widely used for biotransformation.



1

2 R = H
3 R = OH
4 R = OAc

5 R = H
6 R = OH

7 $R^1 = R^2 = H$
8 R^1 = Ts R^2 = H
9 R^1 = H R^2 = OH

10

11

12

Table 1 Hydroxylation of aromadendrane derivatives by *Mucor plumbeus*

Substrate	Product (hydroxyaromadendrane derivative)	Yield (%)
Aromadendr-7(15) -ene (**1**)	7α,14,15-trihydroxy-(**10**)	4
7α-Hydroxy- aromadendrane (**2**)	7α,14-dihydroxy-(**5**)	58
	5α,7α-dihydroxy-(**3**)	1
	6β,7α,14-trihydroxy-(**6**)	1
7α,15-Dihydroxy- aromadendrane (**7**)	7α,14,15-trihydroxy-(**10**)	61
	5α,7α,15-trihydroxy-(**9**)	0.6

Hydroxylation of 7α-hydroxyaromadendrane (**2**) (globulol) gave 7α,14-dihydroxyaromadendrane (**5**) as the major product, identical[6] with the material obtained using *C. aphidicola*. The stereochemistry of the hydroxylation of the *gem*-dimethyl group followed from the NOE (5.2%) observed between the CH_2OH (δ_H 3.26 and 3.36) and the cyclopropane H-2 and H-4 (δ_H 0.66 and 0.75). The location of the hydroxy groups in the minor products followed from their ^{13}C NMR spectra (see Table 2). The magnitude of the coupling constants for the C*H*OAc resonance in **4** (triplet, *J* 9.4 Hz, of doublets, *J* 2.8 Hz) corresponding to two diaxial and one axial : equatorial coupling, established the stereochemistry at C-5. The other minor hydroxylation product had a C*H*(OH) signal at δ_H 3.49 (double doublet, *J* 11.2 and 1.8 Hz). This multiplicity and changes in the ^{13}C NMR spectrum, including a significant *γ-gauche* upfield shift for C-15, were in accord with the C-6β configuration.

Osmylation of aromadendr-7(15)-ene (**1**) gave predominantly one glycol (**7**). The primary alcohol was converted into the unstable mono-toluene-*p*-sulfonate (**8**) and reduced with lithium aluminium hydride to give globulol (**2**) rather than 7-epiglobulol, thus establishing the C-7 stereochemistry in the glycol. The epoxide (**11**) was obtained as a by-product from the reaction with toluene-*p*-sulfonyl chloride. Incubation of the glycol **7** with *M. plumbeus* gave the triol **10** as the major product. The position of the additional hydroxy group at C-14 followed from changes in the ^{13}C NMR spectrum. Furthermore, irradiation of the ^1H NMR signal, δ_H 1.00, produced NOE enhancements at δ_H 3.20 and 3.31 of 1.3 and 1.5%. Irradiation at these resonances produced NOE enhancements of the cyclopropyl H-2 and H-4 signals of 5.2 and 5.4%. This triol was also obtained by incubation of the glycol with *C. aphidicola*. The minor product possessed ^1H and ^{13}C NMR data consistent with its formulation as 5α,7α,15-trihydroxyaromadendrane (**9**).

In these biotransformations the cyclopropane ring remains intact despite hydroxylation taking place at an adjacent position. Although the cleavage of an adjacent cyclopropane ring is taken as a probe for a radical reaction,[8] there are a growing number of examples of biological hydroxylations in which this does not take place.[9] Secondly although the structures of cedrol (**12**) and 7α-hydroxyaromadendrane (**2**) are, at first sight, quite different, when they are drawn such that C-8 of cedrol and C-7 of the aromadendrane are superimposed, the major sites of hydroxylation (C-3 in cedrol, C-14 in the aromadendrane) are co-incident. The formation of the triol **10** from **1** may occur *via* the epoxide **11**.

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Table 2 ^{13}C NMR data for some aromadendrane derivatives, determined in $CDCl_3$ at 125 MHz

	Compound							
Carbon	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**
C-1	39.6	39.9	39.9	38.7	39.3	38.1	38.4	37.2
C-2	28.3	27.9	27.6	24.9	24.6	28.6	27.8	24.9
C-3	19.3	19.7	20.0	26.3	27.1	19.5	19.9	26.5
C-4	26.7	34.2	31.2	23.5	20.7	26.5	33.8	23.4
C-5	20.1	66.9	69.8	19.7	27.7	19.6	66.2	19.2
C-6	44.6	54.4	51.6	44.1	79.2	37.5	47.6	37.2
C-7	75.3	73.4	73.4	75.2	78.1	76.3	74.5	76.1
C-8	57.0	55.8	55.7	56.5	53.3	55.5	54.3	55.1
C-9	26.1	26.0	25.8	25.9	25.2	25.9	25.7	25.7
C-10	34.6	34.3	34.3	34.5	34.8	34.6	34.4	34.6
C-11	36.3	36.5	36.4	36.3	36.4	36.2	36.3	36.2
C-12	16.0	15.4	15.5	15.9	15.9	15.7	15.4	15.7
C-13	15.8	15.7	15.7	11.5	11.6	15.7	15.5.	11.5
C-14	28.6	28.4	28.1	73.1	73.1	28.3	28.3	73.0
C-15	20.2	20.9	20.6	20.1	13.4	61.4	62.1	61.3
Ac			21.5/170.1					

Experimental

^1H and ^{13}C NMR spectra were determined at 500 and 125 MHz respectively. IR spectra were determined as nujol mulls. Silica gel for chromatography was Merck 9385. Petrol refers to the fraction of bp 60–80 °C. Extracts were dried over sodium sulfate. *Mucor plumbeus* was grown in shake flasks (100 cm^3 medium per 250 cm^3 conical flask) on a medium comprising (per dm^3) glucose (20 g), peptone (5 g), yeast extract (3 g) and potassium dihydrogen phosphate (5 g) and the pH was adjusted to 5.6.

Incubation of 7α-Hydroxyaromadendrane (**2**).—(*a*) The substrate (1 g) in ethanol (10 cm^3) was evenly distributed over 45 shake flasks of 1 day old cultures of *M. plumbeus*. After a further 6 days, the broth was filtered and extracted with ethyl acetate. The extract was dried and the solvent evaporated to give an oily residue (1.35 g) which was chromatographed on silica. Elution with petrol–ethyl acetate (1:1) gave 7α,14-hydroxyaromadendrane (**5**) (620 mg) identified by its ^1H NMR spectrum.[6] Further elution gave 5α,7α-dihydroxyaromadendrane (**3**) (10mg) as an oil, δ_H 0.68 (2 H, m, H-2 and H-4), 0.91 (3 H, *J* 7.0 Hz, H-12), 1.09 (6 H, s, H-13, H-14), 1.18 (3 H, s, H-15), 3.42 (1 H, m, H-5); *m/z* 238 (M^+, $C_{15}H_{26}O_2$) (1%), 220 (5), 205 (71), 202 (13), 187 (10), 177 (21), 161 (15), 159 (15). The sample was acetylated with acetic anhydride in pyridine to give the 5α-monoacetate (**4**) as an oil, δ_H 0.71 (1 H, t, *J* 10 Hz), 0.83 (1 H, t, *J* 10 Hz) (H-2 and H-4), 0.92 (3 H, d, *J* 7 Hz, H-12), 1.10 (6 H, s, H-13, H-14), 1.20 (3 H, s, H-15), 2.04 (3 H, s, OAc), 4.52 (1 H, dt, *J* 2.8 and 9.4 Hz, H-5); *m/z* 220 (M−60) (10%), 205 (8), 202 (21), 187 (18), 177 (32), 162 (24). Further elution with ethyl acetate gave 6β,7α,14-trihydroxyaromadendrane (**6**) (15 mg) as a gum, δ_H 0.69 (1 H, t, *J* 10 Hz), 0.84 (1 H, m) (H-2 and H-4), 0.93 (3 H, d, *J* 7 Hz, H-12), 1.08 and 1.12 (3 H, each, s, H-13 and H-15), 3.30 and 3.35 (each 1 H, d, *J* 10.8 Hz, H-14), 3.49 (1 H, dd, *J* 1.8 and 11.2 Hz, H-6); *m/z* 239 M−15) (1%), 236 (2), 221 (3), 218 (5), 205 (4), 193 (11), 175 (30).

(*b*) Under similar conditions aromadendr-7(15)-ene (**1**) (1 g) gave the starting material (700 mg) and 7α,14,15-trihydroxyaromadendrane (**10**) (40 mg), identified by its ^1H NMR spectrum.

(*c*) Under similar conditions 7α,15-dihydroxyaromadendrane (**7**) (950 mg) gave 7α,14,15-*trihydroxyaromadendrane* (**10**) (620 mg), mp 129–130 °C (Found: C, 70.3; H, 10.3. $C_{15}H_{26}O_3$ requires C, 70.8; H, 10.3%); ν_{max}/cm^{-1} 3583; δ_H 0.60 (1 H, dd, *J* 9.6 and 10.5 Hz, H-2), 0.72 [1 H, t, (9.6 Hz) of d (6.4 Hz), 4-H], 0.84 (3 H, d, *J* 7.1 Hz, H-12), 1.00 (3 H, s, H-13), 3.20 and 3.31 (each 1 H, d, *J* 11 Hz, H-14), 3.49 and 3.54 (each 1 H, d, *J* 11.3 Hz, H-15). This compound was identical to the major metabolite (500 mg) obtained from the incubation of 7α,15-dihydroxyaromadendrane (1.3 g) with *C. aphidicola* for 7 days as described previously.[6] Further elution gave 5α,7α,15-trihydroxyaromadendrane (**9**) (6 mg) as a gum, δ_H 0.69 (2 H, m, H-2 and H-4), 0.91 (3 H, d, *J* 7.1 Hz, H-12), 1.08 and 1.09 (each 3 H, s, H-13 and H-14), 3.44 (1 H, m, H-5), 3.63 (2 H, s, H-15); *m/z* 239 (M−15) (3%), 236 (3), 223 (10), 221 (5), 218 (9), 205 (38), 187 (19).

7α,15-*Dihydroxyaromadendrane* (**7**).—Aromadendrene (950 mg) in *tert*-butyl alcohol (30 cm^3) and water (30 cm^3) was treated with a mixture of potassium hexacyanoferrate(III) (4.5 g), potassium carbonate (1.9 g) and osmium tetroxide (30 mg) for 24 h at room temperature. Aqueous sodium thiosulfate (20 cm^3, 10%) was added and the mixture was stirred for 3 h. The product was recovered in ethyl acetate and chromatographed on silica to give 7α,15-*dihydroxyaromadendrane* (**7**) (900 mg) which crystallized from ethyl acetate as needles, mp 112 °C (Found: C, 75.7; H, 10.8. $C_{15}H_{26}O_2$ requires C, 75.6; H, 10.9%), ν_{max}/cm^{-1} 3348; δ_H 0.56 (1 H, t, *J* 9.3 Hz, H-2), 0.63 (1 H, m, H-4), 0.89 (3 H, d, *J* 7.1 Hz, H-12), 0.94 and 1.01 (each 3 H, s, H-13 and H-14), 3.62 (2 H, s, H-15).

Preparation of Globulol (**2**).—The diol **7** (500 mg) in dry pyridine (10 cm^3) was treated with fresh toluene-*p*-sulfonyl chloride (1 g) at room temperature overnight. The solution was poured into dilute hydrochloric acid and the product recovered in ethyl acetate and chromatographed on silica to give the 15-*toluene*-p-*sulfonate* **8** which crystallized from petrol as cubes, mp 93–94 °C (Found: C, 67.5; H, 8.3. $C_{22}H_{32}O_4S$ requires C, 67.3; H, 8.2%); ν_{max}/cm^{-1} 3559, 1598, 1178; δ_H 0.53 (2 H, m, H-2 and H-4), 0.84 (3 H, s), 0.97 (3 H, s) (H-13 and H-14), 0.86 (3 H, d, *J* 7 Hz, H-12), 2.44 (3 H, s, Ar-M4e), 4.06 (2 H, s, H-15), 7.30 and 7.78 (each 2 H, d, *J* 8 Hz, Ar-H). The toluene-*p*-sulfonate (450 mg) in dry tetrahydrofuran (10 cm^3) was treated with lithium aluminium hydride (400 mg) under reflux for 3 h. The solution was cautiously treated with moist ethyl acetate, acidified with dilute hydrochloric acid and the product recovered in ethyl acetate. The extract was dried over sodium sulfate and the solvent evaporated. The residue was chromatographed on silica to give globulol (160 mg), mp 78–80 °C, identified by its ^1H and ^{13}C NMR spectra and by comparison with an authentic sample. On one occasion the *epoxide* **11** was obtained as an oil from the chromatography of the toluene-*p*-sulfonate (Found: *m/z* (e.i) 200.184. $C_{15}H_{24}O$ requires M_r, 220.183); *m/z* 220 (5%), 205 (15), 189 (25), 187 (10), 177 (65), 159 (25), 147 (50); δ_H 0.59 (1 H, t, *J* 9.3 Hz, H-2), 0.63 (1 H, t, *J* 9.3 Hz, of d, *J* 6.0 Hz, H-4), 0.92 (3 H, d, *J* 7.1 Hz, H-12), 1.00 and 1.03 (each 3 H, s, H-13 and H-14), 2.43 (1 H, dd, *J* 4.4 and 1 Hz), 2.67 (1 H, dd, *J* 4.4 and 2.2 Hz) (each H-15).

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References

1 For a review see: V. Lamare and R. Furstoss, *Tetrahedron*, 1990, **46**, 4109.
2 J. R. Hanson and H. Nasir, *Phytochemistry*, 1993, **33**, 835.
3 H. J. M. Gijsen, J. B. P. A. Wijnberg and Ae. de Groot, *Prog. Chem. Org. Nat. Prod*., 1995, **64**, 149.
4 H. J. M. Gijsen, K. Kanai, G. A. Stork, J. B. P. A. Wijnberg, R. V. A. Orru, C. G. J. M. Seelen, S. M. van der Kerk and Ae. de Groot, *Tetrahedron*, 1990, **46**, 7237.
5 W.-R. Abraham, K. Kieslich, B. Stumpf and L. Ernst, *Phytochemistry*, 1992, **31**, 3749.
6 J. R. Hanson, P. B. Hitchcock and R. Manickavasagar, *Phytochemistry*, 1994, **37**, 1023.
7 M. Miyazawa, T. Uemura and H. Kamecka, *Phytochemistry*, 1994, **37**, 1027.
8 C. J. Suckling, *Angew. Chem*., *Int. Ed. Engl*., 1988, **27**, 537.
9 H. L. Holland, M. J. Chernishenko, M. Conn, A. Munez, T. S. Mancharan and M. A. Zawadski, *Can. J. Chem*., 1990, **68**, 696; J. P. Kutney, J. Wagner and G.M. Hewitt, *Helv. Chim. Acta*, 1994, **77**, 1707.