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THE BIOTRANSFORMATION OF 8-EPICEDROL AND SOME RELATIVES BY *CEPHALOSPORIUM APHIDICOLA*

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Key Word Index—*Cephalosporium aphidicola*; microbiological transformation; sesquiterpenoid; 8-epicedrol; hydroxylation.

Abstract—The microbiological hydroxylation of 8-epicedrol and relatives bearing hydroxyl groups at C-9 and C-15 by *Cephalosporium aphidicola* has been shown to take place predominantly at C-3.

INTRODUCTION

In studies aimed at examining the scope of biosynthetically directed biotransformations by *Cephalosporium aphidicola*, we showed that the sesquiterpenoid cedrol (**1**) was hydroxylated primarily at C-3 [1]. Previous studies on the microbiological hydroxylation of cedrol by *Aspergillus niger* [2] and *Beauveria sulfurescens* [3] had shown that hydroxylation occurred predominantly at C-3, although other organisms [4] were less discriminating in their site of attack. We have now undertaken a further study of the structural scope of the biotransformation by *C. aphidicola* with some relatives of cedrol to assess the directing role of the C-8 hydroxyl group.

RESULTS AND DISCUSSION

The compounds selected for study were 8-epicedrol (**2**), cedran-9α-ol (**13**), cedran-8α,9α-diol (**15**), cedran-8α,15-diol (**20**) and isocedran-15-ol (**22**) which have hydroxyl groups at C-8 or on an adjacent carbon. The substrates were prepared from α- and β-cedrene by literature methods [5].

Incubation of 8-epicedrol (**2**) with *C. aphidicola* for 10 days gave a series of metabolites which were separated by chromatography. The metabolites fell into two series: those retaining the C-8α hydroxyl group and those in which dehydration had taken place to afford the 8(9)-alkene. The location of the additional oxygen functions at C-3 was established from the changes in the ^{13}C NMR spectra (see Table 1) [6, 7]. The multiplicity of the C-3 proton resonances, when compared to previous work [1] enabled the stereochemistry of the alcohols to be assigned (3α-ol: δ_H 3.70, triplet (J = 10 Hz) of doublets (J = 5.4 Hz); 3β-ol: δ_H 4.32, doublet (J = 0.9 Hz) of doublets (J = 5.2 Hz)). In each case the 3β-alcohol was the major metabolite. A mixture of cedr-8-en-3β, 15-diol (**10**) and cedran-3β,8α-diol (**6**) was separated after acetylation and the corresponding alcohols were recovered by hydrolysis. The C-2 methyl group in the 3-ketone underwent epimerization. The epimers were distinguished by the long-range coupling (J = 1.9 Hz) shown between the 2α-proton (δ_H 2.05) in **3** and the 4α-proton (δ_H 2.38, *ddd*, J = 1.9, 12.1 and 19.5 Hz). The epimer **12** (2β-H, δ_H 2.12) showed no long-range coupling.

In several experiments, cedran-9α-ol (**13**) was poorly transformed and most of the starting material was recovered. The minor metabolite was identified as cedran-3β,9α-diol (**14**) from the ^{13}C NMR spectrum and the position of the CH(OH) resonance (δ_H 4.32, *dd*, J = 4.3, 8.0 Hz).

Incubation of cedran-8α,9α-diol (**15**) gave cedran-3β,8α,9α-triol (**16**) as the major metabolite accompanied by a minor amount of cedran-8α,9α,12-triol (**19**). Crude **16** contained some of the 3α-isomer, as shown by its ^{1}H NMR spectrum. The crude triol was purified by acetylation to give the 9α-monoacetate (**18**) and the 3β,9α-diacetate (**17**). Incubation of both cedran-8α,15-diol (**20**) and isocedran-15-ol (**22**) with the fungus also gave the 3β-alcohols (**21** and **23**) as metabolites (6.7% and 3% respectively). These were identified by their ^{1}H and ^{13}C NMR spectra (see Table 1).

As noted previously [1] the organism does not hydroxylate cedrene (**11**), but requires an oxygen function. In spite of the variations in the position of the directing hydroxyl group, oxidation by *C. aphidicola* had taken place predominantly at the C-3β position with minor amounts of transformation at the C-3α position and at the C-12 methyl group. The best yields occurred with a tertiary C-8 alcohol, although these were surprisingly independent of the stereochemistry of the alcohol. A possible explanation, which may accomodate this flexibility, is that the directing interaction between the hydroxyl group in the substrate and the enzyme involves not just

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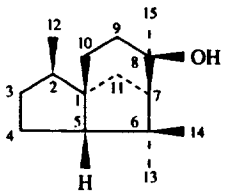

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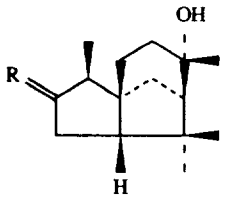
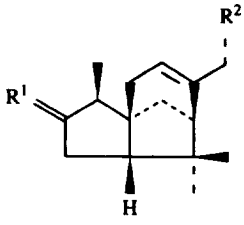
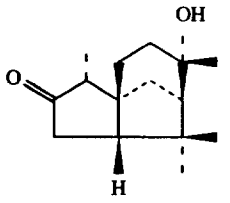


R
2 H_2
3 O
4 α-OH, β-H
5 α-H, β-OAc
6 α-H, β-OH



	R^1	R^2
7	α-H, β-OH	H
8	α-OH, β-H	H
9	α-H, β-OAc	OAc
10	α-H, β-OH	OH
11	H_2	H



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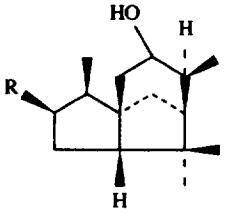
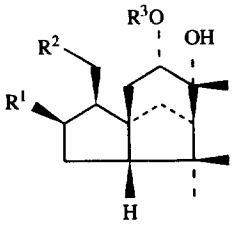
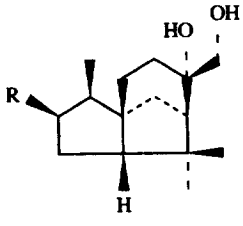


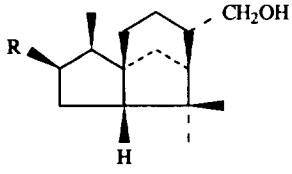
R
13 H
14 OH



15 R^1=R^2=R^3=H
16 R^1=OH, R^2=R^3=H
17 R^1=OAc, R^2=H, R^3=Ac
18 R^1=OH, R^2=H, R^3=Ac
19 R^1=R^3=H, R^2=OH



R
20 H
21 OH



R
22 H
23 OH

a simple bonding directly to the enzyme surface but a hydrogen bonding interaction via water molecules held in a lattice adjacent to the peptide chain of the hydroxylase. This could provide the flexibility of a hydrophilic region rather than a more rigid hydrophilic centre.

EXPERIMENTAL

General experimental details have been described previously [8]. *Cephalosporium aphidicola* (IMI 68689) was grown in shake culture as described previously [8].

Incubation of 8-Epicedrol (**2**). 8-Epicedrol (2 g) in EtOH (25 ml) was evenly distributed among 50 flasks (100 ml medium per flask) of 2-day-old cultures of *C. aphidicola* and the incubation continued for a further 10 days. The metabolites were recovered in EtOAc and separated by chromatography on silica gel. Elution with EtOAc–petrol (3:7) gave sequentially cedr-8-en-3β-ol (**7**) (95 mg), cedr-8-en-3α-ol (**8**) (15 mg), 3-oxocedran-8α-ol (**3**) (80 mg) and 3-oxo-2-epicedran-8α-ol (**12**) (45 mg). Elution with EtOAc–petrol (3:2) gave a mixture followed by cedran-3α,8α-diol (**4**) (31 mg). The mixture was acetylated

Table 1. ^{13}C NMR spectroscopic data

Carbon atom	2	3	4	5	6	8	9	10	12	13
1	53.4	52.0	50.1	52.2	51.9	50.1	51.2	52.3	49.4	54.9
2	41.8	48.4	50.6	45.0	46.2	50.9	39.3	45.0	49.3	41.8
3	36.9	220.1	81.4	82.6	79.5	81.3	81.9	79.6	221.9	36.7
4	25.4	33.6	33.3	31.4	33.7	35.3	30.5	33.4	38.0	25.7
5	56.3	52.8	52.5	54.1	53.9	56.3	57.1	57.1	51.2	58.3
6	41.8	42.6	41.1	41.4	41.3	47.1	47.5	47.5	42.3	44.0
7	61.5	58.6	61.4	62.1	61.8	55.2	52.3	50.1	59.5	55.2
8	73.2	72.2	73.1	73.0	73.0	140.5	138.7	143.7	72.5	46.9
9	34.3	34.6	35.3	34.0	33.9	118.4	123.4	120.2	33.2	73.1
10	30.5	33.1	31.1	31.1	31.5	39.3	40.6	39.7	30.3	46.3
11	39.9	37.7	41.0	40.3	40.3	41.3	44.5	40.9	40.4	43.9
12	15.4	8.9	12.3	9.9	9.4	12.7	10.0	9.5	11.7	15.6
13	29.0	28.1	29.7	27.9	27.9	27.1	25.9	25.9	30.1	27.9
14	28.2	30.2	27.8	29.2	29.1	27.1	27.3	27.4	27.9	28.9
15	30.6	30.7	30.7	30.7	30.5	24.7	68.1	67.0	30.7	17.9
OAc				21.1			21.1, 21.2			
				170.8			170.7, 171.0			

Carbon atom	14	15	16	17	18	19	21	22	23
1	52.6	53.4	52.0	51.8	51.9	52.7	52.3	54.8	53.4
2	45.4	41.6	46.2	44.1	45.8	50.4	46.0	42.2	46.7
3	77.3	36.4	78.5	81.8	79.5	32.1	78.3	37.1	80.0
4	33.2	25.6	34.1	31.3	34.1	26.0	30.8	25.6	34.5
5	54.8	60.8	61.0	60.9	61.0	60.9	56.9	57.9	55.4
6	44.2	40.6	41.1	40.6	41.0	41.4	40.8	43.7	43.2
7	54.6	57.2	54.9	54.5	54.8	58.0	53.6	47.4	47.2
8	46.5	74.3	73.9	72.9	73.4	73.8	74.2	51.5	51.8
9	73.2	72.3	71.4	74.3	74.7	71.5	33.3	33.3	34.0
10	45.5	39.0	39.6	36.4	37.4	39.1	29.3	24.7	24.3
11	42.5	41.4	41.4	38.9	39.2	40.0	39.5	47.2	47.5
12	9.1	15.6	10.0	9.6	9.6	62.6	9.3	15.3	9.5
13	26.7	26.8	27.1	26.5	26.9	27.2	27.4	27.1	27.0
14	28.1	28.7	28.6	27.9	28.4	28.7	28.4	28.6	28.8
15	17.2	29.2	29.4	28.9	29.3	29.4	68.9	67.6	67.5
		20.8, 20.8	21.2						
		170.0, 170.4	170.2						

with Ac_2O–pyridine and separated by chromatography on silica gel with Me_2CO–CH_2Cl_2 (1:9) to give 3β,15-diacetoxycedr-8-ene (**9**) (20 mg) and 3β-acetoxycedran-8α-ol (**5**) (150 mg). These were then treated with 10% (w/v) NaOH–MeOH at room temp. overnight to afford cedr-8-en-3β,15-diol (**10**) (10 mg) and cedran-3β,8α-diol (**6**) (110 mg), respectively. The metabolites were characterized as follows.

Cedr-8en-3α-ol (**7**) was identical to material described previously [1].

Cedr-8-en-3α-ol (**8**). Oil, MS *m/z*: 220 $[M]^+$ ($C_{15}H_{24}O$, 220). IR ν_{max} cm^{-1}: 3450, 1645. NMR δ_H ($CDCl_3$): 0.96 (3H, *d*, *J* = 7 Hz, H-12), 0.99 (3H, *s*, H-14), 1.05 (3H, *s*, H-13), 1.68 (3H, *br s*, H-15), 3.70 (1H, *td*, *J* = 10 and 5.4 Hz, H-3β), 5.19 (1H, *br s*, H-9). Oil, 3-Oxocedran-8α-*ol* (**3**). MS *m/z*: 236 $[M]^+$ ($C_{15}H_{24}O_2$, 236). IR ν_{max} cm^{-1}: 3485, 1735. NMR δ_H ($CDCl_3$): 0.93 (3H, *d*, *J* = 7 Hz, H-12), 0.92 (3H, *s*, H-14), 1.19 (3H, *s*, H-13), 1.32 (3H, *s*, H-15), 2.05 (2H, *m*, H-2, H-5), 2.18 (1H, *dd*, *J* = 5.6 and 19.5 Hz, H-4β), 2.38 (1H, *ddd*, *J* = 1.9, 12.1 and 19.5 Hz, H-4α).

3-Oxo-2-epicedran-8α-ol (**12**). Oil, MS *m/z*: 236 $[M]^+$ ($C_{15}H_{24}O_2$, 236). IR ν_{max} cm^{-1}: 3490, 1735. NMR δ_H ($CDCl_3$): 0.98 (3H, *d*, *J* = 7 Hz, H-12), 1.02 (3H, *s*, H-14), 1.21 (3H, *s*, H-13), 1.35 (3H, *s*, H-15), 2.12 (1H, *q*, *J* = 7 Hz, H-2β), 2.25 (1H, *dd*, *J* = 5.6, 19.5 Hz, H-4β), 2.36 (1H, *dd*, *J* = 12.1, 19.5 Hz, H-4α).

3β,15-Diacetoxycedr-8-ene (**9**). Oil, IR ν_{max} cm^{-1}: 1745. NMR δ_H ($CDCl_3$): 0.83 (3H, *d*, *J* = 7 Hz, H-12), 0.98 (3H, *s*, H-14), 1.04 (3H, *s*, H-13), 2.04 and 2.07 (each

3H, *s*, OAc), 4.42 and 4.48 (each 1H, *d*, $J = 12$ Hz, H-15), 5.23 (1H, *dd*, $J = 4.8$ and 9.8 Hz, H-3α), 5.56 (1H, *br s*, H-9).

3β,15-*Dihydroxycedr*-8-*ene* (**10**). Mp 141°. MS *m/z*: 236 $[M]^+$ ($C_{15}H_{24}O_2$, 236). IR ν_{max} cm^{-1}: 3300, 1650. NMR δ_H ($CDCl_3$): 0.91 (3H, *d*, $J = 7$ Hz, H-12), 0.99 (3H, *s*, H-14), 1.02 (3H, *s*, H-13), 3.96 and 4.05 (1H, *d*, $J = 13.5$ Hz, H-15), 4.35 (1H, *dd*, $J = 5$ and 10 Hz, H-3α), 5.52 (1H, *br s*, H-9).

3β-*Acetoxycedran*-8α-*ol* (**5**). Oil, MS *m/z*: 280 $[M]^+$ ($C_{17}H_{28}O_3$, 280). IR ν_{max} cm^{-1}: 330, 1740. NMR δ_H ($CDCl_3$): 0.82 (3H, *d*, $J = 7$ Hz, H-12), 1.01 (3H, *s*, H-14), 1.15 (3H, *s*, H-13), 1.32 (3H, *s*, H-15), 2.02 (3H, *s*, OAc), 5.26 (1H, *dd*, $J = 4$ and 8.2 Hz, H-3α).

Cedran-3β,8α-*diol* (**6**). Mp 127°. (Found: C, 75.5; H, 11.1. $C_{15}H_{26}O_2$ requires C, 75.6; H, 10.9%.) IR ν_{max} cm^{-1}: 3300. NMR δ_H ($CDCl_3$): 0.89 (3H, *d*, $J = 7$ Hz, H-12), 1.02 (3H, *s*, H-14), 1.16 (3H, *s*, H-13), 1.33 (3H, *s*, H-15), 4.28 (1H, *dd*, $J = 4$ and 8.3 Hz, H-3α).

Cedran-3α,8α-*diol* (**4**). Mp 148°. (Found: C, 75.4; H, 11.0. $C_{15}H_{26}O_2$ requires C, 75.6; H, 10.9%.) IR ν_{max} cm^{-1}: 3300. NMR δ_H ($CDCl_3$): 0.97 (3H, *d*, $J = 7$ Hz, H-12), 1.04 (3H, *s*, H-14), 1.17 (3H, *s*, H-13), 1.33 (3H, *s*, H-15), 3.60 (1H, *td*, $J = 10.6$ and 5 Hz, H-3β).

Incubation of cedran-9α-*ol* (**13**). The alcohol (2 g) was incubated with *C. aphidicola* as above. On chromatography the starting material (1.76 g) was recovered. Further elution gave cedran-3β,9α-diol (**14**) (20 mg), mp 148–150°. (Found: C, 75.4; H, 10.9. $C_{15}H_{26}O_2$ requires C, 75.6; H, 10.9%). IR ν_{max} cm^{-1}: 3300. NMR δ_H ($CDCl_3$): 0.95 (3H, *s*, H-14), 0.98 (3H, *d*, $J = 7$ Hz, H-12), 1.16 (3H, *s*, H-13), 1.23 (3H, *d*, $J = 7$ Hz, H-15), 3.90 (1H, *dd*, $J = 6.5$ and 10 Hz, H-9β), 4.32 (1H, *dd*, $J = 4.3$ and 8 Hz, H-3α).

Incubation of cedran-8α,9α-*diol* (**15**). The diol (2 g) was incubated with *C. aphidicola* as above and the metabolites were separated by chromatography. Elution with EtOAc–petrol (3:2) gave crude cedran-3β,8α,9α-triol (**16**) (1 g) and cedran-8α,9α,12-triol (**19**) (30 mg). Crude **16** was acetylated with Ac_2O–pyridine at room temp. overnight and the acetates purified by chromatography to give 3β,9α-diacetoxycedran-8α-ol (**17**) (250 mg) and 9α-acetoxycedran-3β,8α-diol (**18**) (300 mg). Hydrolysis (10% NaOH–MeOH, room temp., overnight) of the monoacetate (100 mg) gave pure cedran-3β,8α,9α-triol (**16**) (60 mg). The metabolites were characterized as follows.

Cedran-3β,8α,9α-*triol* (**16**). Mp 150°. (Found: C, 68.1; H, 10.6. $C_{15}H_{26}O_3 \cdot 0.5H_2O$ requires C, 68.4; H, 9.9%.) IR ν_{max} cm^{-1}: 3400. NMR δ_H ($CDCl_3$): 0.98 (3H, *d*, $J = 7$ Hz, H-12), 1.02 (3H, *s*, H-14), 1.15 (3H, *s*, H-13), 1.45 (3H, *s*, H-15), 3.85 (1H, *dd*, $J = 6.5$, 10.2 Hz, H-9), 4.35 (1H, *dd*, $J = 4.2$ and 8.5 Hz, H-3α).

3β,9α-*Diacetoxycedran*-8α-*ol* (**17**). Mp 120–124°. (Found: C, 67.1; H, 8.6. $C_{19}H_{30}O_5$ requires C, 67.4; H, 8.9%.) IR ν_{max} cm^{-1}: 3300, 1745. NMR δ_H ($CDCl_3$): 0.70 (3H, *d*, $J = 7$ Hz, H-12), 0.91 (3H, *s*, H-14), 1.09 (3H, *s*, H-13), 1.16 (3H, *s*, H-15), 1.92 and 1.99 (each 3H, *s*, OAc), 4.97 (1H, *dd*, $J = 6.4$ and 10.5 Hz, H-9), 5.13 (1H, *dd*, $J = 3.8$ and 7.8 Hz, H-3).

9α-*Acetoxycedran*-3β,8α-*diol* (**18**). Mp 132–134°. (Found: C, 68.1; H, 9.3. $C_{17}H_{28}O_4$ requires C, 68.9; H, 9.5%)IR ν_{max} cm^{-1}: 3340, 1745. NMR δ_H ($CDCl_3$): 0.91 (3H, *d*, $J = 7$ Hz, H-12), 1.04 (3H, *s*, H-14), 1.21 (3H, *s*, H-13), 1.29 (3H, *s*, H-15), 2.10 (3H, *s*, OAc), 4.29 (1H, *dd*, $J = 4.1$, 8.2 Hz, H-3), 5.10 (1H, *dd*, $J = 6.5$ and 10.6 Hz, H-9).

Cedran-8α,9α,12-*triol* (**19**). Mp 152°. (Found: C, 69.1; H, 10.7. $C_{15}H_{26}O_3 \cdot 0.5\ H_2O$ requires C, 68.4; H, 9.9%.) IR ν_{max} cm^{-1}: 3350. NMR δ_H ($CDCl_3$–pyridine): 1.12 (3H, *s*, H-14), 1.24 (3H, *s*, H-13), 1.60 (3H, *s*, H-15), 3.68 (1H, *dd*, $J = 6.1$, 10.3 Hz, H-9), 3.91 and 3.95 (each 1H, *d*, $J = 7$ Hz, H-12).

Incubation of cedran-8α,15-*diol* (**20**). The diol (1.5 g) was incubated with *C. aphidicola* as above. The metabolites were separated by chromatography to give the starting material and cedran-3β,8α,15-triol (100 mg), mp 123–126°. (Found: C, 68.7; H, 10.2. $C_{15}H_{26}O_3 \cdot 0.5\ H_2O$ requires C, 68.4; H, 9.9%.) IR ν_{max} cm^{-1}: 3300. NMR δ_H ($CDCl_3$): 0.77 (3H, *d*, $J = 7$ Hz, H-12), 0.80 (3H, *s*, H-14), 0.94 (3H, *s*, H-13), 3.45 and 3.54 (each 1H, *d*, $J = 10.8$ Hz, H-15), 4.16 (1H, *dd*, $J = 4$ and 8.2 Hz, H-3α).

Incubation of iso cedran-15-*ol* (**22**). The alcohol (1 g) was incubated with *C. aphidicola* as above. The metabolites were separated by chromatographty to give cedran-3β,15-diol (**23**) (30 mg), mp 111–114°. (Found: C, 75.4; H, 10.9. $C_{15}H_{26}O_2$ requires C, 75.6; H, 10.9%.) IR ν_{max} cm^{-1}: 3667. NMR δ_H ($CDCl_3$): 0.92 (3H, *d*, $J = 7$ Hz, H-12), 0.96 (3H, *s*, H-14), 1.11 (3H, *s*, H-13), 3.66 (2H, *d*, $J = 7$ Hz, H-15), 4.29 (1H, *dd*, $J = 4$ and 8 Hz, H-3α).

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