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THE MICROBIOLOGICAL HYDROXYLATION OF SOME PINANE MONOTERPENOIDS BY *CEPHALOSPORIUM APHIDICOLA*

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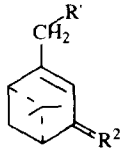
Key Word Index—*Cephalosporium aphidicola*; biotransformation; pinanes; monoterpenoids.

Abstract—The microbiological oxidation of some pinane alcohols at C-4 by *Cephalosporium aphidicola* is described.

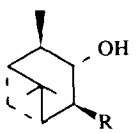
INTRODUCTION

The microbial biotransformation of monoterpenoids has attracted considerable interest both from the mechanistic point of view and as a means of preparing novel perfumery substances [see Ref. [1] and references cited therein]. The rigid structure of the bicyclic pinane monoterpenoids has been used as a framework to study the directing effect of an amide group on microbial hydroxylation by *Beauveria sulfurescens* [2]. The hydroxylation of α-pinene by *Armillariella mellea* has been studied [3] in the context of the role of these metabolites as aggregation pheromones for bark beetles that attack damaged fir trees. In this paper, we report on the biotransformation of some derivatives of α-pinene by *Cephalosporium aphidicola*. These were studied in order to examine the effect of the position of a hydroxyl substituent on the site of microbial biotransformation. The following compounds were examined in which the position of a potentially directing hydroxyl group was systematically varied around part of the cyclic structure: nopol (**1**), myrtenol (**4**), nopinol (**8**), isopinocampheol (**10**) and *trans*-verbenol (**6**).



	R^1	R^2
1	CH_2OH	H_2
2	CH_2OH	α-H, β-OMe
3	CH_2OH	α-H, β-OH
4	OH	H_2
5	OH	α-H, β-OH
6	H	α-H, β-H
7	H	O

8 R = α-OH, β-H
9 R = O



10 R = H
11 R = OH

RESULTS AND DISCUSSION

The substrates were incubated with *C. aphidicola* on shake culture for 12 days. Most of the cultures did not grow well after the addition of the monoterpenoid substrate. The metabolites produced are tabulated in Table 1. The structures of the metabolites were established from the changes in the ^{13}C NMR spectra when compared to the starting materials (Table 2), [4, 5]. Nopinone (**9**) and verbenone (**7**) were identified by comparison with authentic samples. The stereochemistry of hydroxylation in **2**, **3** and **5** followed from n.O.e. studies. In particular irradiation of the $-C-CH_3$ signal [δ_H0.85 in **2**; δ_H0.86 in **3**; δ_H0.79 in **5**] led to enhancements (5.6%, 3.0% and 7.1%) of both the new C<u>H</u>(OH) signal and (0.8% and 1.1%) of the alkene =C<u>H</u> signal, whereas irradiation of the $-C-CH_3$ signal (δ_H1.33, 1.33 and 1.27, respectively) only enhanced overlapping alkane multiplets. Hence the new hydroxyl group is *trans* to the dimethyl bridge. The product from isopinocampheol (**11**) was identical to the product of hydroboronation of verbenone (**7**) [6] but of opposite optical rotation. Incubation of α-pinene with the medium alone for 10 days did not give any detectable transformation products.

It is interesting to note that the site of hydroxylation of these pinane derivatives did not appear to be immediately related to their hydroxylation pattern. It is possible that as the size of the substrate becomes smaller than the size of the iron–haem complex, the major determining

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Table 1. Metabolites of pinane monoterpenoids

Substrate	Products	Yield (%)
Nopol (**1**)	4β-Methoxynopol (**2**)	6
	4β-Hydroxynopol (**3**)	11
Myrtenol (**4**)	4β,10-Dihydroxypin-2-ene (**5**)	23
Nopinol (**8**)	Nopinone (**9**)	18
Isopinocampheol (**10**)	3α,4β-Dihydroxypinane (**11**)	11
Verbenol (**6**)	Verbenone (**7**)	61

feature of the site of microbiological hydroxylation becomes access to the particular hydrogen atoms for abstraction and is less dominated by more distant enzyme–substrate interactions.

EXPERIMENTAL

General experimental details. IR: nujol mulls or thin films; ^{1}H and ^{13}C NMR: $CDCl_3$ on a Bruker WH 360 spectrometer. Extracts were dried over sodium sulphate. Silica for chromatography was Merck 9385.

Biotransformations. Cephalosporium aphidicola (IMI 68689) was grown as described previously [7]. The substrate (2 g) in EtOH (25 cm^3) was evenly distributed between 50 shake flasks (100 cm^3 medium per flask) and the fermentation was continued for a further 12 days. The mycelium was filtered and washed with EtOAc. The broth which was obtained was extracted with EtOAc. The combined extracts were dried and the solvent was evaporated to give a residue which was chromatographed on silica gel. The metabolites were eluted with increasing concentrations of EtOAc in petrol (60–80°).

(a) Biotransformation of nopol (**1**) gave (i) 4β-methoxynopol (**2**) (64 mg) as an oil. $[\alpha]_D$ −93°, M^+ 196.145 ($C_{12}H_{20}O_2$ requires 196.146). ν_{max} 3392 cm^{-1}; δ_H0.85 (3H, *s*, 8-H), 1.33 (3H, *s*, 9-H), 3.35 (3H, *s*, OMe), 3.63 and 3.64 (each 1H, *t*, J = 7 Hz, 11-H), 3.82 (1H, *t*, J = 3 Hz, 4α-H), 5.48 (1H, *m*, 3-H). Irradiation at δ_H5.48 collapsed the signal at δ_H3.82 to a doublet; (ii) 4β-hydroxynopol (**3**) (179 mg), mp 112–113°, $[\alpha]_D$ −127° (found: C, 72.1; H, 10.0; $C_{11}H_{18}O_2$ requires C, 72.5; H, 10.0%). ν_{max} 3262 cm^{-1}; δ_H0.86 (3H, *s*, 8-H), 1.33 (3H, *s*, 9-H), 3.63 and 3.64 (each 1H, *t*, J = 7 Hz, 11-H), 4.29 (1H, *t*, J = 3.1 Hz, 4α-H), 5.43 (1H, *m*, 3-H), irradiation at δ_H5.43 collapsed the signal at δ_H4.29 to a doublet.

(b) Biotransformation of myrtenol (**4**) gave (−)-4β,10-dihydroxypin-2-ene (**5**) (207 mg), mp 130–133°, $[\alpha]_D$ −168° (Found: C, 71.3; H, 9.6; $C_{10}H_{16}O_2$ requires C, 71.4; H, 9.6%). ν_{max} 3262, 1653 cm^{-1}; δ_H0.79 (3H, *s*, 8-H), 1.27 (3H, *s*, 9-H), 3.88 and 3.91 (each 1H, *d*, J = 14.5 Hz, 10-H), 4.24 (1H, *t*, J = 1.5 Hz, 4α-H), 5.46 (1H, *m*, 3-H). Irradiation of the signal at δ_H5.46 led to the collapse of the signal at δ_H4.24 to a doublet.

(c) Biotransformation of nopinol (**8**) (800 mg) gave nopinone (**9**) (140 mg) as an oil. ν_{max} 1713 cm^{-1}; δ_H0.85 (3H, *s*, 8-H), 1.33 (3H, *s*, 9-H) identified by comparison (^{1}H NMR) with authentic material.

(d) Biotransformation of isopinocampheol (**10**) gave 3α,4β-dihydroxypinane (**11**) (74 mg), mp 150° (lit. [6], 152°), $[\alpha]_D$ +19°(Found: C, 70.1; H, 10.7, calc. for $C_{10}H_{18}O_2$: C, 70.5; H, 10.7%). ν_{max} 3320 cm^{-1}; δ_H1.04 (3H, *s*, 8-H), 1.13 (3H, *s*, *d*, J = 6.2 Hz, 10-H), 1.22 (3H, *s*, 9-H), 3.90 (2H, *m*, 3- and 4-H). A sample prepared by hydroboronation of verbenone (**7**) [6] had identical IR and ^{1}H NMR spectra but a rotation of −17.4°.

(e) Biotransformation of verbenol (**6**) gave verbenone (**7**) (1.1 g) as an oil. ν_{max} 1680, 1618 cm^{-1}; δ_H1.01 (3H, *s*, 8-H), 1.34 (3H, *s*, 9-H) 2.02 (3H, *s*, 10-H), 5.73 (1H, *br s*, 3-H), identified by comparison with an authentic sample.

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Table 2. ^{13}C NMR data for the pinane derivatives

C	1	2	3	4	5	6	7	8	9	10	11
1	45.5	46.6	47.1*	43.3	43.8	47.6	49.6	48.3	57.9	47.4	48.2
2	144.6	148.7	148.7	147.7	151.1	147.1	169.6	73.6	214.3	47.7	48.7
3	118.7	118.4	121.0	117.7	117.6	119.3	121.0	28.4	32.7	71.3	80.2
4	31.5	79.2	70.2	31.6	69.6	73.4	202.6	24.8	21.4	38.8	81.9
5	40.5	43.2	46.7*	40.9	47.1	48.1	57.4	41.0	40.4	41.7	44.3
6	31.2	29.1	28.8	31.1	28.4	35.4	40.5	26.0	25.2	34.3	31.9
7	37.7	45.5	46.2	37.9	46.0	38.8	53.5	37.5	41.0	38.1	38.0
8	21.0	20.7	20.7	21.0	20.3	22.5	22.0	22.7	22.1	23.6	24.3
9	26.1	26.6	26.5	26.1	26.3	26.8	26.5	27.4	25.8	27.6	28.4
10	40.1	39.8	39.6	65.8	64.2	22.5	23.3			20.6	19.6
11	59.9	59.9	59.9								
OMe		56.1									

*Assignments may be interchanged.

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