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THE BIOTRANSFORMATION OF AMBROX® AND SCLAREOLIDE BY *CEPHALOSPORIUM APHIDICOLA**

JAMES R. HANSON† and ALMAZ TRUNEH

School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ, U.K.

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Key Word Index—*Cephalosporium aphidicola*; biotransformation; diterpenoid; Ambrox®, aphidicolin.

Abstract—The biotransformation of Ambrox® and sclareolide by the fungus, *Cephalosporium aphidicola*, has been examined. The substrates were hydroxylated at C-3β and C-6β while Ambrox® was also oxidized at C-12 in a xenobiotic rather than a biosynthetically patterned fashion.

INTRODUCTION

The late stages of the biosynthesis of the diterpenoid, aphidicolin (**2**) by the fungus, *Cephalosporium aphidicola*, involve a series of hydroxylations of the biosynthetic intermediate, aphidicolan-16β-ol (**1**) [1]. We have been interested in seeing to what extent these hydroxylations are reflected in the microbiological transformation of analogues of biosynthetic intermediates [2]. The perfumery compound (−)-Ambrox® (**3**) [3] possesses a formal structural relationship to rings A–C of aphidicolan-16β-ol (**1**). Its biotransformation and that of its relative sclareolide (**7**) [4] by *C. aphidicola* were therefore of interest in the context of establishing the breadth of the substrate: specificity of the enzymes involved in the biosynthesis of the fungal metabolite, aphidicolin.

RESULTS AND DISCUSSION

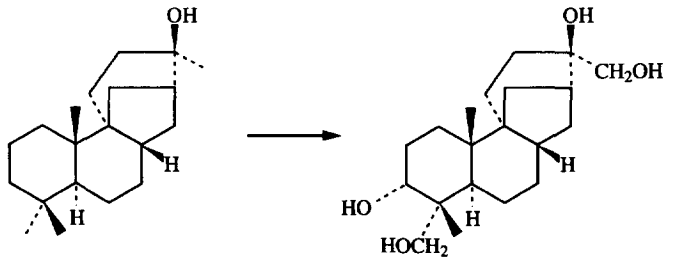
The major metabolites from the incubation of Ambrox (**3**) with *C. aphidicola* for 10 days in shake culture were the 3β-alcohol **4**, the 3β,12-diol (isolated as the butyl ether) **5**, the 3β, 6β-diol **6** and the 3β,6β-dihydroxylactone **10**. The location of the additional oxygen functions at C-3, C-6 and C-12 followed from changes in the ^{13}C NMR spectra (see Table 1). In particular, the methylene resonances associated with C-3 and C-6 were replaced by CH-O- signals while the adjacent signals showed downfield shifts consistent with the insertion of a hydroxyl group. There were γ-upfield shifts on a number of carbon signals including C-1, C-18, C-19 and C-5 arising from the hydroxylation at C-3. As anticipated, the oxidation at C-12 affected the signal assigned to C-11. The stereochemistry of the additional hydroxyl groups followed from the multiplicity of the C<u>H</u>(OH) signals in the ^{1}H NMR spectrum (H-3, δ_H 3.20, *dd*, $J = 5$ and 11 Hz; H-6, δ_H 4.6, narrow *t*, $J = 2.8$ Hz). The formation of the butyl ether **6** as an artefact arises because butanol was used in the extraction. The parent hemi-acetal was detected although it was not obtained in pure form.

Incubation of sclareolide (**7**) gave a comparable range of products. These included the known [5] 3-ketone **8**, the 3β-alcohol **9** and the 3β,6β-dihydroxylactone **10**. The positions of the oxygen functions were established from the ^{13}C NMR spectra (see Table 1) while the stereochemistry of the hydroxyl group in **9** followed from the multiplicity of the H-3 ^{1}H NMR signal, [δ_H 3.27, *dd*, $J = 5$ and 11 Hz]. The metabolism of Ambrox and sclareolide by *C. aphidicola* was not particularly clean and a number of mixtures were obtained from the chromatography, suggesting that epimers were being formed. On one occasion a C-8 epimer (**11**) was isolated. It is known [6] that Ambrox isomerizes at C-8 under acidic conditions and this isomerization may have occurred during the extraction.

In conclusion, although the fungus hydroxylated these substrates, the pattern of hydroxylation is that of xenobiotic transformation. The stereochemistry of hydroxylation at C-3 is equatorial rather than axial as in aphidicolin biosynthesis while there is a marked absence of hydroxylation on the methyl groups. Although hydroxylation at the C-6β position is found in the metabolism of aphidicolin, it is also found in the metabolism of steroids by this organism. The results of these biotransformations contrast with those observed with sclareol (**12**) which possesses more of the carbon skeleton associated with aphidicolin intermediates. In

*Ambrox® is a registered trademark of Firmenich S.A.

†Author to whom correspondence should be addressed.



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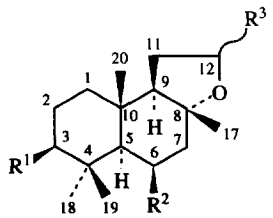
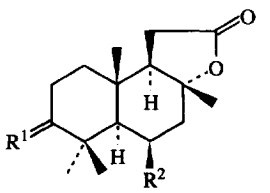
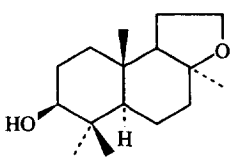


3 $R^1 = R^2 = R^3 = H$
4 $R^1 = OH$, $R^2 = R^3 = H$
5 $R^1 = OH$, $R^2 = H$, $R^3 = O\text{-}Bu$
6 $R^1 = R^2 = OH$, $R^3 = H$



7 $R^1 = H_2$, $R^2 = H$
8 $R^1 = O$, $R^2 = H$
9 $R^1 = \alpha\text{-}H, \beta\text{-}OH$, $R^2 = H$
10 $R^1 = \alpha\text{-}H, \beta\text{-}OH$, $R^2 = OH$



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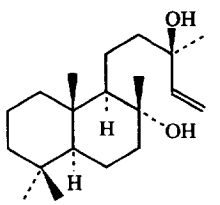

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particular, it possesses a side-chain hydroxyl group that, when superimposed over that of aphidicolan-16β-ol, juxtaposes rings A and B of sclareol over those of aphidicolin. This suggests that the 16-hydroxyl group of aphidicolin function as a biosynthetic directing group.

Table 1. ^{13}C NMR Data for the metabolites of Ambrox® and sclareolide, determined in $CDCl_3$

C	3	4	5*	6†	7	8	9	10‡	11
1	39.9	38.1	37.9	39.7	39.5§	37.7	37.6	39.3	37.8
2	18.4	27.1	27.1	26.5	18.1	33.4	26.8	26.4	27.3
3	42.4	78.9	79.0	78.9	42.1	215.5	78.6	78.7	78.1
4	33.1	38.7	38.7	39.2	33.1	47.3	38.8	39.4	38.7
5	57.2	55.9	55.7	57.1	56.6	54.3	55.3	56.9	55.2
6	20.6	20.4	20.2	68.3	20.5	21.4	20.2	67.7	20.3
7	39.7	39.5	39.8	47.3	38.7§	37.7	38.4	46.4	44.2
8	79.9	79.8	80.9	79.6	86.4	85.6	86.1	86.4	71.8
9	60.1	59.9	60.1	60.4	59.1	58.1	58.8	59.4	59.4
10	36.2	35.9	35.8	35.9	36.0	35.5	35.7	35.6	39.1
11	22.6	22.6	30.8	22.6	28.7	28.6	28.7	28.7	28.2
12	65.0	64.9	104.4	64.3	176.9	175.9	176.5	177.4	63.6
17	21.1	21.0	23.4	21.5	21.5	21.1	21.5	22.3	24.6
18	33.6	28.2	28.1	27.1	33.1	26.6	27.8	26.8	28.4
19	21.1	15.1	15.2	16.3	20.9	20.7	15.1	16.3	15.7
20	15.0	15.1	15.1	15.9	15.0	14.5	15.1	16.0	15.5

*n-Butanol 13.9, 19.4, 31.9, 68.2. †Solvent $CDCl_3$–MeOH, ‡Solvent $CDCl_3$–DMSO.
§May be interchanged.

EXPERIMENTAL

General experimental details have been described previously [7]. Ambrox was obtained from Aldrich as ambroxide. Sclareolide was also obtained from Aldrich.

Incubation of Ambrox (**3**). The substrate (**3**) (2 g) in DMSO–EtOH (5:1, 30 ml) was evenly distributed between 50 flasks of a 4-day-old shake culture of *C. aphidicola*. After a further 10 days, the broth was filtered and extracted with EtOAc (×2) and EtOAc–BuOH (1:1) (×1). The combined extracts were dried over Na_2SO_4 and the solvent was evapd. The residue was chromatographed on silica gel. The column was eluted with a gradient of EtOAc–petrol starting with 5% EtOAc and finishing with pure EtOAc. 3β-hydroxyambroxide (7β-hydroxyperhydro-3a,6,6,9a-tetramethylnaphtho- [2,1b]-furan) (**4**) (360 mg) had mp 191–192°. MS *m/z*: 253 $[M+1]^+$, $C_{16}H_{28}O_2$ requires 252; 237 $[M-15]^+$, 219 $[M-15-18]^+$; IR $\nu_{max} \times cm^{-1}$: 3583; ^1H NMR δ_H ($CDCl_3$): δ 0.80, 0.84, 1.00 and 1.09 (each 3H, *s*), 3.23 (1H, *dd*, $J = 5$ and 11 Hz, H-3), 3.83 and 3.90 (each 1 H,*m*, H-12). 12-*n*-Butoxy-3β-hydroxyambroxide (**5**) (174 mg), mp 109–110°, $[M]^+$ 324, ($C_{20}H_{36}O_3$ requires 324); IR $\nu_{max} \times cm^{-1}$: 3582, 3484 (br); ^1H NMR δ_H ($CDCl_3$): δ 0.79, 0.87 (each 3H, *s*), 0.91 (3H, *t*, $J = 7.4$ Hz), 0.99 and 1.25 (each 3H, *s*), 3.22 (1H, *dd*, $J = 5.4$ and 10.9 Hz, H-3), 3.37 and 3.73 (each 1H, *t.t*, $J = 6.4$ and 9.4 Hz, O–CH_2–) 5.10 (1H, *dd*, $J = 5.2$ and 6.0 Hz, H-12). 3β,6β-Dihydroxyambroxide (**6**) (131 mg), mp 209–211, $[M]^-$ 268 ($C_{16}H_{28}O_3$ requires 268), 253 $[M-15]^+$; IR $\nu_{max} \times cm^{-1}$: 3380 (*br*); ^1H NMR δ_H($CDCl_3$): 1.10, 1.17, 1.18 and 1.33 (each 3H, *s*), 3.20 (1H, *dd*, $J = 5.2$ and 10.8 Hz), 3.82 (1H, *d*, $J = 8.2$ Hz), 3.95 (1H, *t*, $J = 8.2$ of *d*, $J = 3$ Hz) (H-12), 4.60 (1H, *t*, $J = 2.5$ Hz, 6-H). 3β,6β-Dihydroxysclareolide (**10**) (178 mg), mp 227–230° (Found: C, 67.9; H, 9.3. $C_{16}H_{26}O_4$ requires C, 68.1; H, 9.3%); IR $\nu_{max} \times cm^{-1}$: 3583, 3266 (*br*), 1738; ^1H NMR δ_H ($CDCl_3$): δ 1.17, 1.18, 1.23 and 1.59 (each 3H, *s*), 3.21 (1H, *dd*, $J = 5$ and 11 Hz, H-3), 4.66 (1H, *t*, $J = 3.0$ Hz, H-6). On one occasion 3β-hydroxy-8-epiambroxide (**11**) (148 mg), mp 200–203°, IR $\nu_{max} \times cm^{-1}$ 3520 (*br*); ^1H NMR: δ 0.82, 0.84, 1.05 and 1.24 (each 3H, *s*), 3.25 (1H, *dd*, $J = 5$ and 11 Hz), 3.56 and 3.84 (each 1H, *m*) (H-12) was isolated from the chromatography.

Incubation of sclareolide. Sclareolide (**7**) (1.5 g) in DMSO–EtOH (5:1, 25 cm^3) was evenly distributed between 50 flasks of a 4-day-old culture of *C. aphidicola*. The fermentation was harvested after a further 10 days. The metabolites were recovered in EtOAc–*n*-butanol and sepd by CC on silica using a gradient of EtOAc–petrol to give 3-oxosclareolide (**8**) (355 mg), mp 175–177° (lit., [5] 184–186°), 3β-hydroxysclareolide (**9**) (563 mg), mp 155–158° (lit., [5] 163–164°) and 3β,6β-dihydroxysclareolide (**10**) (382 mg), mp 230° which were identified by their ^1H and ^{13}C NMR spectra.

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