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THE HYDROXYLATION OF GLOBULOL AND 7-EPIGLOBULOL BY *CEPHALOSPORIUM APHIDICOLA*

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Key Word Index—*Cephalosporium aphidicola*; globulol; 7-epiglobulol; sesquiterpenoid; microbiological hydroxylation.

Abstract—Globulol and 7-epiglobulol were shown to be hydroxylated by *Cephalosporium aphidicola* on one (C-14) of the methyl groups geminal to the cyclopropane ring in 48.5 and 56% yield, respectively. The significance of this hydroxylation adjacent to a cyclopropane ring is noted.

INTRODUCTION

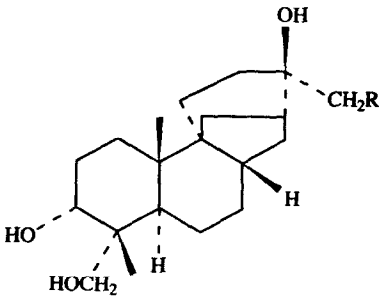
The structural requirements for molecular recognition for biosynthetically directed microbiological transformations need to be defined for a number of pathways. An efficient step (52.6% incorporation) in the biosynthesis of aphidicolin (**2**) involves the hydroxylation of 3α,16β,18-trihydroxyaphidicolane (**1**) at C-17 [1]. This step involves the conversion of a $>C(OH)\cdot Me$ group to a glycol $>C(OH)\cdot CH_2OH$. Since this is a potentially useful bioconversion, we have investigated the biotransformation of compounds possessing the $>C(OH)\cdot Me$ moiety attached to fragments reminiscent of the C/D ring system of aphidicolin (**2**) [2]. The aromadendrane sesquiterpenoids, globulol (**3**) and 7-epiglobulol (**5**) [3, 4] possess this $>C(OH)\cdot Me$ unit attached as epimers to a seven-membered ring which can be superimposed on rings C and D of aphidicolin. We have therefore investigated their biotransformation by *Cephalosporium aphidicola*, the organism that produces aphidicolin.

The microbiological hydroxylation of globulol (**3**) by *Diplodia gossypina* and *Bacillus megaterium* has been reported in previous studies on the biotransformation of sesquiterpenoids [5, 6].

RESULTS AND DISCUSSION

Globulol (**3**) and epiglobulol (**5**) were separately incubated with *C. aphidicola* on shake culture for six days. In each case a single major metabolite was formed (48.5 and 56% conversion, respectively) as well as aphidicolin. The metabolites, $C_{15}H_{26}O_2$, each contained a primary alcohol [δ3.28 and 3.35, each 1H, doublet, $J = 10.8$ Hz] which replaced one of the C-methyl groups. However, neither compound behaved as a 1,2-glycol and reacted with periodate. Furthermore in the ^{13}C NMR spectra (Table 1), the signal from the quaternary carbon of the cyclopropane ring showed a significant downfield shift ($\Delta\delta$7.2 ppm). Hence one of the geminal cyclopropyl methyl groups had been hydroxylated. The site of hydroxylation was established by NOE studies. Irradiation of the primary alcohol ^1H NMR signals in the product from globulol [δ3.28 and 3.35] produced NOE enhancements of the cyclopropyl proton resonances [δ0.67 and 0.77] of 6.2 and 6.7%, respectively, implying a *cis* relationship. The irradiation also produced NOE enhancement (6%) of the methyl group signal at δ1.10. When this NOE experiment was applied to the hydroxylation product of 7-epiglobulol (**6**), the cyclopropyl signals (δ0.64 and 0.72) were enhanced (5.1 and 5.6%, respectively) on irradiation of the primary alcohol signals. There was also a 1.6% enhancement of the methyl group signal (δ1.16). Consequently the structures **4** and **6** were assigned to the metabolites. The ^{13}C NMR spectra (Table 1) were consistent with these structures. The metabolite from 7-epiglobulol gave crystals which were suitable for X-ray crystallography and this confirmed the structure as **6** (Fig. 1). In particular this demonstrated that the cyclopropane ring had remained intact. The diol **4** was obtained previously in the biotransformation of globulol by *D. gossypina* and *B. megaterium* [6].

Although the initial objective was not fulfilled, there are several interesting features which emerge from these results. The facile opening of a cyclopropane ring has been used as a probe for the intervention of free radical intermediates in enzymatic hydroxylation and other reactions [7, 8]. However, in this case ring opening has not taken place suggesting either that the radical is constrained or that it reacts sufficiently quickly for ring opening not to take place. A number of other instances have been recorded [9] in which ring opening does not occur. Secondly, there does not appear to be any stereochemical directing effect from the 7-hydroxyl group in that the same exo-methyl group is efficiently hydroxylated in both epimers.



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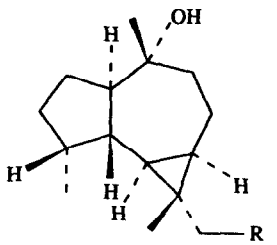
2 R=OH



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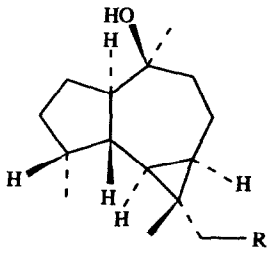
4 R=OH



5 R=H
6 R=OH

EXPERIMENTAL

General experimental details. IR spectra were determined as nujol mulls; ^1H and ^{13}C NMR spectra were determined for solns in $CDCl_3$ at 500 and 125 MHz, respectively. Extracts were dried over Na_2SO_4; silica for chromatography was Merck 9385. Petrol refers to the fr. bp 60–80°. Globulol was purchased from Fluka whilst 7-epiglobulol was a generous gift from Prof. Ae de Groot.

Incubation of globulol (**3**). *Cephalosporium aphidicola* (IMI 68689) was grown on shake culture (100 ml medium per 250 ml flask) for 3 days as described previously [2]. Globulol (**3**) (1.5 g) in DMSO (25 ml) was evenly distributed amongst 50 flasks and the fermentation continued for 6 days. The mycelium was removed by filtration and the broth extracted with EtOAc. The extract was dried, the solvent evapd and the residue chromatographed on silica gel. Elution with EtOAc–petrol (1:1) gave 7β,14-dihydroxyaromadendrane (**4**) (780 mg), mp 115–117° (lit. [6] 113°) (Found: C, 75.7; H, 10.7. calc. for $C_{15}H_{26}O_2$: C, 75.6; H, 11.0%). IR ν_{max} cm^{-1}: 3308. ^1H NMR: δ0.92 (3H, *d*, J = 7.1 Hz, H-12), 1.10 (3H, *s*, H-13), 1.12 (3H, *s*, H-15), 3.28 and 3.55 (each 1H, *d*, J = 10.8 Hz, H-14). Further elution with EtOAc gave aphidicolin (**2**) (93 mg).

Incubation of 7-epiglobulol (**5**). *Cephalosporium aphidicola* was grown in shake culture as above for 3 days. Epiglobulol (1.2 ml) in DMSO (25 ml) was evenly distributed between 50 flasks and the fermentation was then continued for a further 6 days. The mycelium was filtered and the broth was extracted with EtOAc. The extract was dried and the solvent evapd to give a residue that was chromatographed on silica gel. Elution with EtOAc–petrol (1:1) gave 7α,14-dihydroxyaromadendrane (**6**) (720 mg), mp 125–128°. (Found: C, 75.4; H, 11.0. $C_{15}H_{26}O_2$ requires C, 75.6; H, 11.0%). IR ν_{max} cm^{-1}:

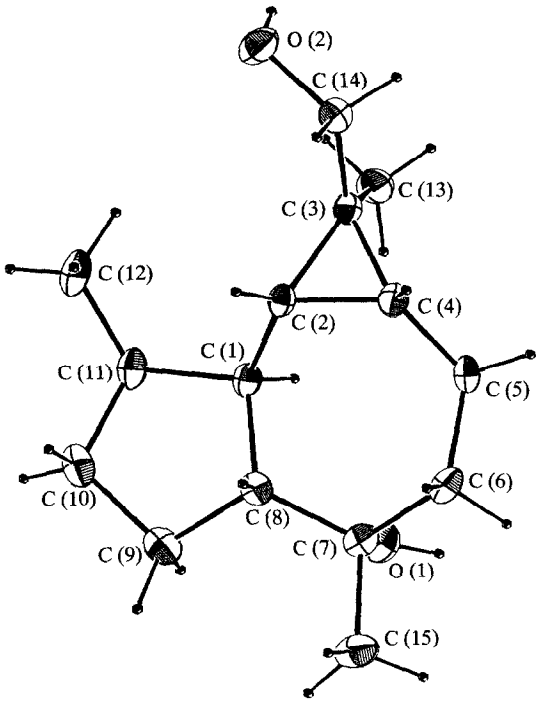


Fig. 1. X-Ray crystal structure for 7α,-14-dihydroxyaromadendrane (**6**).

Table 1. ^{13}C NMR signals for globulol, 7-epiglobulol and metabolites determined in $CDCl_3$ at 125 MHz

C	3	4	5	6
1	39.6	38.7	37.5	36.5
2	28.3	25.0	28.7	25.5
3	19.3	26.5	20.5	27.7
4	26.7	23.5	27.0	23.9
5	20.1	19.7	19.1	18.6
6	44.6	44.2	42.8	42.5
7	75.3	75.2	72.2	72.2
8	57.0	56.6	55.9	55.5
9	26.1	25.9	26.5	26.4
10	34.6	34.5	34.6	34.5
11	36.3	36.3	35.7	35.8
12	16.0	15.9	16.6	16.5
13	15..8	11.5	15.8	11.5
14	28.6	73.4	28.8	73.6
15	20.2	20.2	31.1	31.2

3343. ^{1}H NMR: δ0.93 (3H, *d*, $J=7.1$ Hz, H-12), 1.16 (3H, *s*, H-13), 1.21 (3H, *s*, H-15), 3.27 and 3.35 (each, 1H, *d*, $J=10.8$ Hz, H-14). Further elution with EtOAc gave aphidicolin (**2**, 60 mg).

Crystallographic data and structure determination of **6**. $C_{15}H_{26}O_2$, M_r 238.4, tetragonal, space group $P4_32_12$ (No. 96), $a=8.628(4)$, $b=8.628(4)$, $c=38.118(8)$ Å, $\alpha=\beta=\gamma=90°$, $U=2837.5$ Å^3, $Z=8$, $D_{calc}=1.12$ g cm^{-3}, $F(000)=1056$, monochromated MoK$_\alpha$ radiation, $\lambda=0.71069$ Å, $\mu=0.7$ cm^{-1}.

Data were collected using a crystal *ca* 0.3 × 0.3 × 0.2 mm on an Enraf–Nonius CAD4 diffractometer in the $\theta-2\theta$ mode. A total of 2914 reflections were measured for $2<\theta<25°$ and h, 0→10, k, 0→10 and l, 0→45°. There were 2532 unique reflections and 1792 significant reflections with $|F^2|>2\sigma(F^2)$ were used in the refinement where $\sigma(F^2)=\{\sigma^2(I)+(0.04I)^2\}^{\frac{1}{2}}/L_p$. There was no crystal decay and no correction was made for absorption.

The structure was solved by direct methods using SHELXS-86 and the non-hydrogen atoms were refined anisotropically using the Enraf–Nonius MoLEN programs. The H atoms at C-12 were fixed at calcd positions using $U_{iso}=1.3U_{eq}$ for the parent atom whilst the other H_2 atoms were freely refined isotropically. The absolute structure was chosen as that known on chemical grounds. With the weighting scheme of $W=\sigma^{-2}(F)$, the refinement converged with $R=0.050$ and $R'=0.051$. All crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

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