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BIOTRANSFORMATION OF *ENT*-16β,19-DIHYDROXYKAURANE BY *CEPHALOSPORIUM APHIDICOLA*

JAMES R. HANSON, PETER B. HITCHCOCK and JACQUELINE A. TAKAHASHI

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

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Key Word Index—*Cephalosporium aphidicola*; fungus; diterpenoid; microbiological hydroxylation; aphidicolin.

Abstract—The major product of hydroxylation of *ent*-16β,19-dihydroxykaurane by *Cephalosporium aphidicola* is the 11β-alcohol. The structure was established by spectroscopic methods and X-ray crystallography.

INTRODUCTION

The biosynthesis of aphidicolin (**3**) by *Cephalosporium aphidicola* involves the stepwise hydroxylation of aphidicolan-16β-ol (**1**) at C-18, C-3 and C-17 [1]. 16β, 18-Dihydroxyaphidicolane (**2**) is an efficient intermediate on this pathway. The further transformation of aphidicolin (**3**) by the fungus includes hydroxylation at C-6 and C-11 [2]. The biotransformation of the *ent*-kaurane analogue of **2**, *ent*-16β,19-dihydroxykaurane (**4**), by this organism was of interest in the context of the borderline between the analogue and the xenobiotic biotransformation of diterpenoids [3].

The substrate, compound **4** had been prepared [4] previously by the hydration of *ent*-19-hydroxykaur-16-ene (**5**) and its stereochemistry had been assigned by analogy with the hydration of *ent*-kaur-16-ene. The C-16 epimer has been prepared [5] by the reaction of methyl magnesium iodide with *ent*-19-hydroxy-17-norkauran-16-one.

RESULTS AND DISCUSSION

The substrate was prepared by epoxidation of *ent*-kaur-16-en-19-oic acid (**6**) with *m*-chloroperbenzoic acid and reduction of the methyl ester (**8**) of the epoxide with lithium aluminium hydride. The epoxidation was only stereoselective and reduction of the crude ester gave one major and three minor products. The stereochemistry of the 16-alcohols (**4**) (major) and (**9**) (minor) was confirmed by a series of NOE experiments. Irradiation of the 20-H resonances (δ_H 1.01 in **4**; δ_H 0.94 in **9**) produced NOE enhancements (8 and 8.9%) of the 14α-H signals at δ_H 1.89 in **4** and δ_H 1.87 in **9**. Decoupling experiments led to the identification of the 14β-H resonances (J = 12.1 Hz), at δ_H 1.56 in **4** and δ_H 1.05 in **9**. There were long-range couplings between the 14α-H and resonances at δ_H 1.55 in **4** and δ_H 1.49 in **9**, which were assigned to the 15β-H. The 14β- and 15-H resonances overlap in **4**. Irradiation of the 17-H produced NOE enhancements of signals at δ_H 1.55 (4.1%; 15β-H) in **4** and δ_H 1.05 (4.6%; 14β-H) and δ_H 1.42 3.1%; 15α-H) in **9**. This led to the assignment of the stereochemistry of **4** and **9** as shown. Furthermore, the downfield shift (0.51 ppm) of the 14β-H in **4** compared to **9**, arising from the interaction of the hydroxyl group at C-16, is also in accord with this assignment. The other minor products were identified as the 15α,16α-epoxide (**10**) [δ_H 2.65, 15-H; 1.46, 17-H_3] and the 16α,17,19-triol (**11**) [δ_H 3.69 and 3.71, doublets, J = 11 Hz; δ_H 3.65 and 3.98, doublets, J = 11 Hz; 17- and 19-H_2].

Incubation of the major diol (**4**) with *Cephalosporium aphidicola* afforded two metabolites. The changes in the ^{13}C NMR spectrum (Table 1) of the major metabolite, $C_{20}H_{34}O_3$ (**12**), when compared to the substrate, suggested that it was an 11-hydroxylation product. Irradiation at δ_H 0.97 (20-H) in **12** produced an NOE enhancement of 4% at δ_H 4.09 (11-H) in accordance with the structure **12**. Furthermore, as a consequence of the interaction between the 11β-hydroxyl group and C-17, the NMR signal for 17-H appeared at δ_H 1.92 (pyridine-d_5 solution). However, the 11α-H resonance appeared only as a doublet (J = 7 Hz). Consequently, the structure of this metabolite was confirmed by X-ray crystallography (Fig. 1). The ^{13}C NMR spectrum of the minor, less polar metabolite, $C_{20}H_{32}O_2$ (**14**) also showed that there was an oxygen function at C-11. The 11-H resonance was a narrow triplet (δ_H 4.34, J = 3.2 Hz) and this signal received an NOE enhancement (0.7%) on irradiation of the 20-H signal (δ_H 1.07). The ^{13}C NMR resonances (Table 1) were very similar, particularly for rings C and D, to those for the 11β,16β-ether (**13**) [6]. The conformational changes to ring C brought about by the 11β,16β-epoxide formation are reflected in the positions of a number of the signals compared to unbridged relatives.

Although this biotransformation did not show hydroxylations at C-3 or C-17 characteristic of aphidicolin

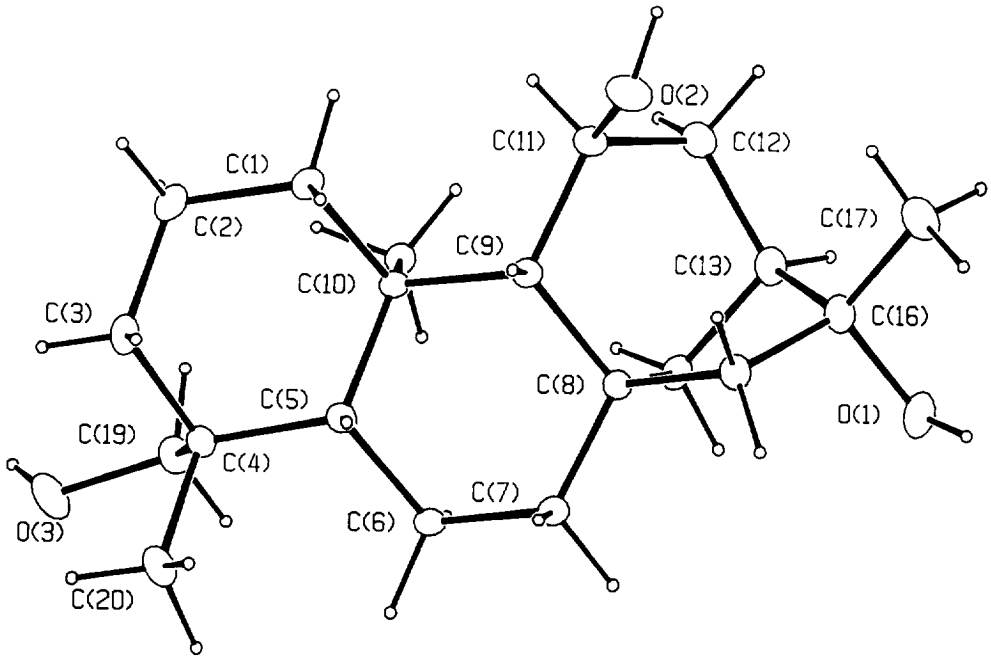
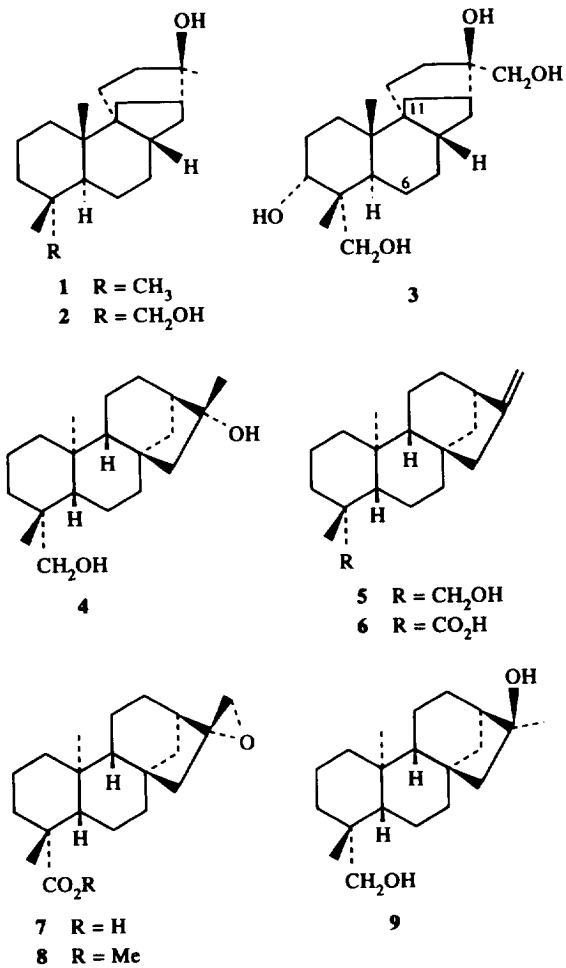
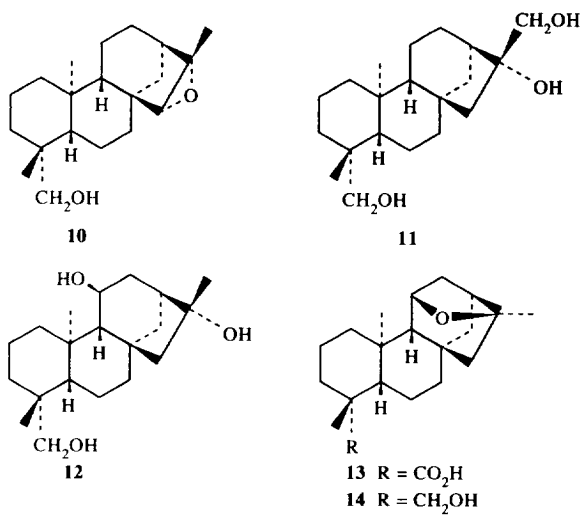


Fig. 1. X-ray crystal structure of *ent*-11α,16β,19-trihydroxykaurane (**12**).





biosynthesis, the hydroxylation at C-11 is found in the further transformation of aphidicolin by this organism. The ether **14** may be an artefact or it might arise from a small amount of the 16-epimeric alcohol **9** present in the initial substrate **4**.

EXPERIMENTAL

General experimental details. ^{1}H and ^{13}C NMR spectra were determined at 500 and 125 MHz respectively: IR spectra were determined as major mulls; silica for column chromatography was Merck 9385; petrol refers to light petroleum bp 60 – 80°. *Cephalosporium aphidicola* (IMI 68689) was grown on the medium de-

Table 1. ^{13}C NMR data for *ent*-16β,19-dihydroxykaurane and its relatives (determined in $CDCl_3$ at 125 MHz)

Carbon atom	4	9	10	12*	13†	14
1	40.4	40.2	40.5	40.2	40.6	40.3
2	18.2	18.5	18.1	18.7	19.6	18.1
3	35.6	35.3	35.5	36.2	38.5	35.5
4	38.6	38.4	39.0	38.6	43.6	38.5
5	56.7	56.6	56.5	57.0	57.4	57.2
6	20.6	19.9	19.2	20.4	22.2	20.1
7	42.4	42.3	36.7	43.4	41.6	41.0
8	45.2	44.1	43.6	44.3	45.1	44.8
9	57.0	57.1	50.6	65.1	58.6	59.2
10	39.2	39.2	38.6	39.4	37.3	36.7
11	18.0	18.0	18.0	65.1	76.8	76.8
12	26.7	26.4	26.9	37.7	38.5	38.3
13	49.0	46.6	39.1	49.6	45.9	45.5
14	37.5	38.3	32.0	37.2	43.4	43.7
15	57.8	56.9	68.3	56.4	57.1	57.1
16	79.3	78.8	61.3	78.8	85.6	85.5
17	24.4	31.8	14.5	26.2	23.5	23.2
18	27.0	26.8	27.0	28.0	29.4	26.8
19	65.5	64.5	65.3	64.2	180.2	64.5
20	18.2	17.9	17.9	18.5	17.8	17.9

*In pyridine-d_5.
†In pyridine-d_5 (taken from ref. [6]).

scribed previously [7] except that shake culture (100 ml medium in 250 cm^3 conical flasks) were used.

Prepn of ent-16β,19-*Dihydroxykaurane* (**4**). *ent*-Kaur-16-en-19-cic acid (300 mg) in $CHCl_3$ (15 ml) was treated with *m*-chloroperbenzoic acid (300 mg) at 0°. After 30 min. at room temp., aq. Na_2SO_3 was added, the soln was washed with aq. $NaHCO_3$ and H_2O and dried. The solvent was evapd and the product was purified by column chromatography on silica gel in $CHCl_3$–EtOAc (9:1) to give, after careful recrystallization, compound **7**, mp 157–161° (Found: C, 75.2; H, 9.5. $C_{20}H_{30}O_3$ requires C, 75.4; H, 9.5%). ^1H NMR ($CDCl_3$): δ 0.96 (3H, *s*, 20-H), 1.25 (3H, *s*, 18-H), 2.82 and 2.89 (each 1H, *d*, J = 4.7 Hz, 17-H). The above crude epoxide (1 g) in EtOH (50 ml) was treated with CH_2N_2 in Et_2O until the yellow colour persisted. The solvent was evapd and the product (**8**) crystallized from EtOAc. The epoxide (**8**) (650 mg) in THF (50 ml) was treated with LAH (300 mg) under N_2 and heated under reflux for 1 hr. The excess of reagent was destroyed with EtOAc, H_2O and dil. HCl, and the product were recovered in EtOAc. Column chromatography on silica gel using a gradient of EtOAc in petrol gave compound **10** (57 mg), mp 149–151° (Found: C, 78.35; H, 10.55. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%). ^1H NMR ($CDCl_3$): δ 0.96 (3H, *s*, 20-H), 0.98 (3H, *s*, 19-H), 1.41 (3H, *s*, 17-H), 2.65 (1H, *s*, 15-H), 3.40 and 3.70 (each 1H, *d*, J = 11 Hz, 19-H). Further elution gave compound **9** (65 mg), mp 219° (lit. [5], 233–234°). (Found: C, 78.4; H, 11.0 Calc. for $C_{20}H_{34}O_2$: C, 78.4; H, 11.2%). ^1H NMR ($CDCl_3$; CD_3OD): δ 0.86 (3H, *s*, 19-H), 0.94 (3H, *s*, 20-H), 1.23 (3H, *s*, 17-H), 3.30 and 3.66 (each 1H, *d*, J = 11 Hz, 19-H). Further elution **4** (437 mg), mp 199° (lit. [4] 200–201°) (Found: C, 78.3; H, 11.2. Calc. for $C_{20}H_{34}O_2$: C, 78.4; H, 11.2%). ^1H NMR ($CDCl_3$): δ 0.96 (3H, *s*, 20-H), 1.02 (3H, *s*, 18-H), 1.36 (3H, *s*, 17-H), 3.44 and 3.73 (each 1H, *d*, J = 10.9 Hz, 19-H). Finally, elution with EtOAc gave compound **11** (22 mg), mp 228–229° (lit. [4], 224–225°). ^1H NMR (pyridine-d_5): δ 1.15 and 1.19 (each 3H, *s*, 18- and 20-H), 3.65 and 3.98 (each 1H, *d*, J = 1 Hz), 3.69 and 3.71 (each 1H, *d*, J = 11 Hz) (17- and 19-H).

Incubation of ent-16β,19-*Dihydroxykaurane* (**4**) *with* C. aphidicola. *ent*-16β,19-Dihydroxykaurane (300 mg) and CCC (150 mg) in EtOH (25 ml) were evenly distributed between 25 flasks of *C. aphidicola* on day 3 after inoculation. After a further 6 days the broth was acidified and extracted with EtOAc and the metabolites were separated by column chromatography on silica gel using a gradient of EtOAc in petrol to give **14** (18 mg) as a gum. MS *m/z*: 304 $[M - 18]^+$, IR ν_{max}. 3540 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.97 and 1.07 (each 3H, *s*, 18- and 20-H), 1.34 (3H, *s*, 17-H), 3.45 and 3.79 (each 1H, *d*, J = 11 Hz, 19-H) and 4.34 (1H, *t*, J = 3.2 Hz, 11-H). Further elution gave **12** (30 mg) as prisms, mp 240–242°. (Found: C, 70.1; H, 10.3. $C_{20}H_{34}O_3 \cdot H_2O$ requires C, 70.5; H, 10.05%). IR ν_{max}. 3550 cm^{-1}, ^1H NMR (pyridine-d_5): δ 0.97 (3H, *s*, 20-H), 1.14 (3H, *s*, 18-H), 1.92 (3H, *s*, 17-H), 3.57 and 3.91 (each 1H, *d*, J = 11 Hz, 19-H), 4.09 (1H, *d*, J = 7 Hz, 11-H).

Crystallographic structure determination. $C_{20}H_{34}O_3 \cdot H_2O$, M 340.5, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 7.302(1), b = 11.351(2), c = 21.994(7) Å, U = 1823.0 $Å^3$, Z = 4, $D_{calc.}$ = 1.24 g cm^{-3}, F(000) 752, monochromated Mo-K_α radiation λ = 0.71069 Å, μ = 0.8.

Data were collected using a crystal *ca* 0.35 × 0.3 × 0.25 mm on an Enraf–Nonius CAD 4 diffractometer operating in the θ–2θ mode. A total of 2535 unique reflections were measured with $2 < \theta < 2\theta$ for *h*, 0–9, *k*, 0–15 and *l*, 0–29 and 2136 reflections with $|F^2| > 2\sigma(F^2)$ were used in the refinement where $\sigma(F^2) = \{\sigma^2(I) + (0.04\ I)^2\}^{1/2}/L_p$. There was no crystal decay and no correction was made for absorption.

The structure was solved by direct methods using SHELXS-86, and non-hydrogen atoms were refined anisotropically by full matrix least squares using programs from the Enraf–Nonius MoLEN package. The hydrogen atoms were freely refined isotropically. With a weighting scheme of $w = 1/\sigma^2(F)$, the refinement converged with R = 0.042 and R' = 0.046. The absolute configuration was chosen to correspond with that known from chemical evidence. The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

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