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16β,17-DIHYDROXYAPHIDICOLAN-18-OIC ACID, A MINOR DITERPENOID METABOLITE OF *CEPHALOSPORIUM APHIDICOLA*

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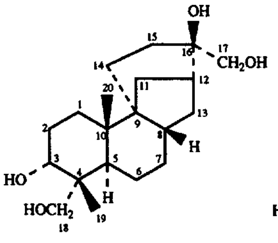
Key Word Index—*Cephalosporium aphidicola*; fungus; diterpenoid; aphidicolin.

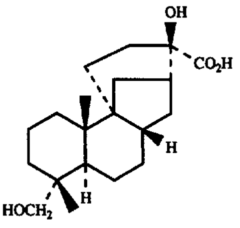
Abstract—The structure of 16β,17-dihydroxyaphidicolan-18-oic acid, a minor metabolite of *Cephalosporium aphidicola*, has been established by X-ray crystallography. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The tumour inhibitory diterpenoid, aphidicolin (**1**) is produced by the fungus, *Cephalosporium aphidicola* [1]. During the preparation of quantities of aphidicolin for chemical transformations, we have isolated a number of minor metabolites related to aphidicolin [2, 3]. These included the acid, 16β,18-dihydroxyaphidicolan-17-oic acid (**2**) [3]. We have now isolated an isomeric acid by chromatography of some aphidicolin residues.



1



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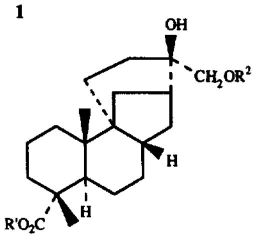

3 R' = R² = H
4 R' = Me, R² = H
5 R' = Me, R² = CHO

RESULTS AND DISCUSSION

The new metabolite **3**, $C_{20}H_{32}O_4$, had spectroscopic properties which characterized the oxygen functions as a carboxylic acid, a tertiary alcohol and a primary alcohol [ν_{max} 3583, 3400 (br), 1688 cm^{-1}; δ_C 182.6, CO_2H; 74.4; C-OH; 67.8, δ_H 3.21 and 3.33, each doublets, $J = 11$ Hz, CH_2OH]. Methylation of the acid with caesium fluoride and methyl iodide in dimethylformamide [4] gave a separable mixture of the methyl ester **4** and the formate **5** confirming the presence of a primary alcohol and carboxylic acid in the metabolite. The formate still retained the hydroxyl absorption of the tertiary alcohol. The ^{13}C NMR spectra of the acid and its derivatives (see Table 1) were similar to that of 16β,18-dihydroxyaphidicolan-17-oic acid (**2**). However, in the ^{1}H NMR spectrum there was no nuclear Overhauser enhancement (NOE) of the primary alcohol signals (δ_H 3.21 and 3.33) on irradiation of either C-Me signals (δ_H 0.80 or 1.07). However, there were mutual enhancements of the methyl group signals (4% at δ 1.07 on irradiation at δ 0.80 and 3% at δ 0.80 on irradiation at δ 1.07). This led to a probable structure of 16β,17-dihydroxyaphidicolan-18-oic acid (**3**) for the metabolite. However, there was also the possibility that the stereochemistry might not be identical (e.g. at C-16) with that of aphidicolin and hence the structure was confirmed by X-ray crystallography of the methyl ester (see Fig. 1).

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were determined at 500 and 125 MHz, respectively; IR spectra were determined as nujol mulls. Silica for chromatography was Merck 9385.

Isolation of compound **2**. Chromatography of the

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Table 1. ^{13}C NMR signals of compounds **3**, **4**, and **5** determined at 125 MHz in $CDCl_3$

C	3	4	5
1	38.0	37.2	37.2
2	18.9	18.3	18.3
3	35.7	33.2	33.1
4	39.9	39.6	39.6
5	41.6	39.8	39.9
6	26.6	26.1	26.0
7	27.2	26.7	26.6
8	40.5	40.8	41.0
9	49.6	48.8	48.8
10	47.6	47.6	47.5
11	33.0	32.6	32.4
12	41.6	41.5	41.3
13	31.6	31.1	31.2
14	25.3	24.7	24.4
15	28.7	29.6	28.6
16	74.4	74.5	73.1
17	67.8	67.7	69.1
18	182.6	179.8	179.6
19	17.2	16.6	16.6
20	15.2	15.0	14.9
OMe		51.4	51.8
OCHO			181.6

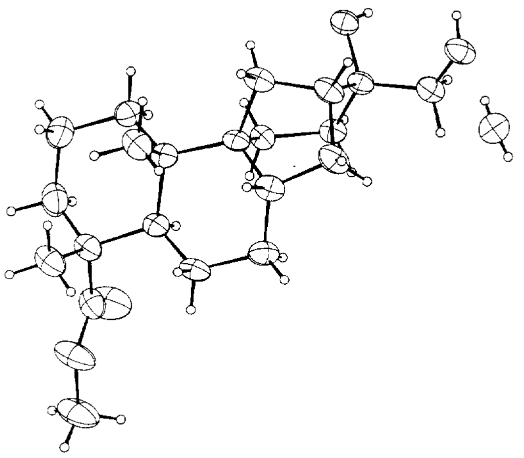

Fig. 1. X-Ray Crystal Structure of Compound **4**.

residues (3 g) from the crystallization of aphidicolin (10 g) on silica gave in the frs eluted with EtOAc–petrol (4:1) 16β,17-dihydroxyaphidicolan-18-oic acid (210 mg) which crystallized from EtOAc–petrol as needles, mp 233.5–235°. (Found: C, 71.2; H, 9.5. $C_{20}H_{32}O_4$ requires C, 71.4; H, 9.6%) IR ν_{max} cm^{-1}: 3583, 3450 (br), 1688. ^{1}H NMR ($CDCl_3$–pyridine-d_5): δ 0.80 (3H, *s*, H-19), 1.07 (3H, *s*, H-20), 3.21 and 3.33 (each 1H, *d*, J = 11 Hz. H-17).

Methylation with caesium fluoride and methyl iodide. The acid (160 mg) in dry DMF (4 ml) was treated with dry caesium fluoride (310 mg) and MeI (320 mg) at room temp. for 24 hr. The soln was poured into H_2O and the product recovered in EtOAc. The extract was dried over Na_2SO_4 and the solvent evapd to give a gum which was chromatographed on silica. Elution with EtOAc–petrol (3:7) gave methyl 17-formyloxy-16β-hydroxyaphidicolan-18-oate (**5**) (22 mg) which crystallized from EtOAc–petrol as needles, mp 143°. (Found: C, 69.9; H, 9.1. $C_{22}H_{34}O_5$ requires C, 69.8; H, 9.0%). IR ν_{max} cm^{-1}: 3583, 1722 (br). ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, *s*, H 19), 1.12 (3H, *s*, H-20), 3.58 (3H, *s*, OMe), 3.99 (2H, *br s*, H-17), 8.06 (1H, *s*, OCHO). Further elution with EtOAc–petrol (1:1) gave methyl 16β,17-dihydroxyaphidicolan-18-oate (**4**) (56 mg) which crystallized from EtOAc–petrol as needles, mp 129–131°. (Found: C, 70.2; H, 9.7. $C_{21}H_{34}O_4$. 0.5 H_2O requires C, 70.2; H, 9.8%) IR ν_{max} cm^{-1} 3580, 3274, 1719, ^{1}H NMR ($CDCl_3$): δ 0.99 (3H, *s*, H-19), 1.19 (3H, *s*, H-20), 3.39 and 3.42 (each 1H, *d*, J = 11 Hz, H-17), 3.65 (3H, *s*, OMe).

Crystallographic data and structure determination. $C_{21}H_{34}O_4$. 0.5 H_2O, M_r 359.49, orthorhombic, space group $C222_1$ (no 20), $a = 7.808(4)$, $b = 10.958(7)$, $c = 46.702(12)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 3996(3)$ Å^3, $Z = 8$, D_{calc} 1.20 g cm^{-3}, $F(000)$ 1576, monochromated Mo-Kα radiation $\lambda = 0.71073$ Å, $\mu = 0.08$ mm^{-1}.

Data were collected using a crystal size *ca* $0.40 \times 0.30 \times 0.30$ mm on an Enraf-Nonius CAD4 diffractometer. A total of 1990 unique reflections were measured for $2 < \theta < 25°$ and $0 < h < 9$, $0 < k < 12$, $0 < l < 55$. 1403 Reflections with $I > 2\sigma(I)$ were used in the refinement. There was no crystal decay and no absorption correction was applied.

The structure was solved by direct methods using SHELXS 86 and SHELXL 93. The non-hydrogen atoms were refined anisotropically by full matrix least squares. Hydrogen atoms were included in riding mode with U_{iso} (H) = $1.2U_{eq}$ (C) or $1.5U_{eq}$ (C) for methyl groups. Hydroxyl groups were fixed at idealised geometry but with the torsion angle defining the hydrogen atom position, refined with U_{iso} (H) equal to $1.5U_q$ (O). There was a molecule of H_2O lying on a crystallographic 2-fold rotation axis for which the hydrogen atom was fully refined. The final R indices were $R_1 = 0.055$, $wR_2 = 0.138$ and R indices (all data) $R_1 = 0.085$, $wR_2 = 0.175$. The tables of crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

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