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TWO C_{10} LACTONES FROM *CEPHALOSPORIUM APHIDICOLA*

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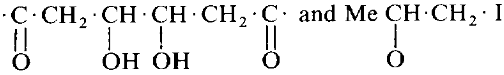
Key Word Index—*Cephalosporium aphidicola*; fungus; cephalosporolide G; diplodialide B; lactones.

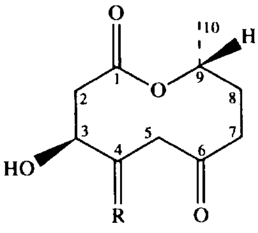
Abstract—Cephalosporolide G, diplodialide B and *Z*-3-methylpent-2-en-1,5-dioic acid have been obtained from the fungus, *Cephalosporium aphidicola*.

INTRODUCTION

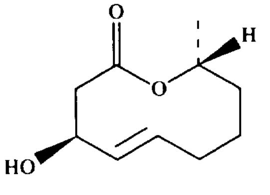
The fungus, *Cephalosporium aphidicola*, produces a number of 10-membered ring lactones, known as the cephalosporolides [1, 2]. The diplodialides from *Diplodia pinea* [3], the pyrenolides from *Pyrenophora teres* [4] and the decarestrictines from *Penicillium simplicissimum* and *P. carylophilum* [5] belong to the same class of compound. Some of these lactones are inhibitors of various stages in steroid biosynthesis. In this report, we describe the isolation of a further novel member of this series, cephalosphorolide G (**1**) and the known diplodialide B (**3**) from *C. aphidicola*.

RESULTS AND DISCUSSION

Cephalosporolide G (**1**), $C_{10}H_{16}O_5$, is an isomer of cephalosporolide C (**2**) [2]. The ^{13}CNMR spectrum (Table 1) showed similarities to the other cephalosporolides. Spin decoupling studies in the ^1HNMR spectrum established the presence of the fragments  $\cdot\underset{O}{\underset{\|}{C}}\cdot CH_2\cdot\underset{OH}{\underset{|}{CH}}\cdot\underset{OH}{\underset{|}{CH}}\cdot CH_2\cdot\underset{O}{\underset{\|}{C}}\cdot$ and $Me\,\underset{O}{\underset{|}{CH}}\cdot CH_2\cdot$ Irradiation of the doublet (J = 11.5 Hz) of triplets (J = 2.5 Hz) at δ4.34 removed a small coupling (J = 2.5 Hz) from the other CH(OH) signal at δ4.41 and also from a doublet (J = 17 Hz) of doublets (J = 2.5 Hz) at δ2.52. A large coupling (J = 11.5 Hz) was removed from a double-doublet (J = 11.5 and 17 Hz) at δ2.83. Irradiation at the CH(OH) signal, δ4.41 (*ddd*, J = 2.5, 5 and 10.5 Hz) removed a 2.5 Hz coupling from the signal at δ4.34, a 5 Hz coupling from δ2.73 and a 10.5 Hz coupling from δ2.64. The latter signals formed an AB system (J = 18 Hz). Irradiation of the signal at δ5.09 showed that it was coupled to two one-proton multiplets at 2.05 and 2.10, and to the methyl group doublet (δ1.25, J = 6.4 Hz). Bearing in mind the presence of a carbonyl group, this leads to the overall structure (**1**). The stereochemistry of cephalosporolide C (**2**) has been established by X-ray crystallography [2]. Comparison of the ^1HNMR coupling constants, $J_{3:4}$ and $J_{4:5}$, leads to the stereochemistry (**1**) from cephalosporolide G. This is the same stereochemistry as found in thiobiscephalosporolide A [1].



1 R α-H, β-OH
2 R α-OH, β-H



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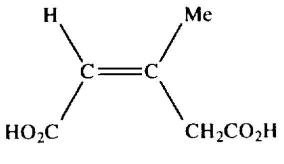

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Table 1. ^1H and ^{13}CNMR data for the C_{10} lactones **1** and **3**

	1		**3**	
C	^{13}C	^1H	^{13}C	^1H
1	169.7	—	171.0	—
2	35.9	2.52, 2.83	35.3	2.55, 2.61
3	67.2	4.34	68.0	4.66
4	69.9	4.41	132.8	5.60
5	46.1	2.64, 2.73	128.2	5.68
6	209.7	—	44.8	1.95, 2.30
7	40.4	2.35	32.5	n.a.
8	33.9	2.05, 2.08	35.3	n.a.
9	72.1	5.09	72.8	4.86
10	19.5	1.25	21.7	1.18

These C_{10} lactones are often produced by fermentations which give an oily extract and fail to produce much aphidicolin. Diplodialide B (**3**) was isolated from the oily residues of a group of such fermentations. It was identified by its NMR spectra (Table 1). It has previously been described [3] as an oil, but in this work it was recrystallized as its 3, 5-dinitrobenzoate.

The more polar fractions from the fermentation contained a dicarboxylic acid, *Z*-3-methylpent-2-en-1,5-dioic acid (**4**) [6]. The stereochemistry of the double bond of this acid was confirmed by NOE experiments. Irradiation of the methyl group resonance produced an enhancement of 2% at δ3.64 ($= C \cdot CH_2 \cdot CO$) and 7.8% at δ5.86 ($HC = C$), whilst irradiation at the alkene resonance (δ5.86) produced a 2% enhancement of the methyl group signal (δ1.98). The formation of this compound may represent a 'dumping' mechanism for un-utilized hydroxymethyl-glutaryl co-enzyme A. 6-Hydroxymethyleugenin [7] was also isolated from these fermentations.

EXPERIMENTAL

General. ^{1}H NMR were determined at 360 or 500 MHz. ^{13}C NMR spectra at 125 MHz, for solns in $CDCl_3$. IR were recorded in Nujol mulls. Silica for chromatography was Merck 9385. Petrol refers to the fr. bp 60–80°.

Isolation of metabolites. Cephalosporium aphidicola (IMI 68689) was grown in shake culture (100 ml per 250 ml conical flask) on a medium as described previously [8] for 12 days. The broth (5 l) was extracted with EtOAc and the metabolites sepd by chromatography on silica gel. Elution with EtOAc–petrol (1:9) gave ergosterol (73 mg) identified by its IR, UV and ^{1}H NMR spectra. Elution with EtOAc–petrol (1 :4) gave 6-hydroxymethyleugenin [7] identified by its IR and ^{1}H NMR spectra. Elution with EtOAc–petrol (7:3) gave cephalosporolide G (68 mg), (**1**), mp 165–166°. (Found: C, 56.1; H, 7.4. $C_{10}H_{16}O_5$ requires C, 55.6; H. 7.5%), IR ν_{max} cm^{-1}: 3300, 1725, 1703, ^{1}H and ^{13}C NMR: in Table 1. Further elution with EtOAC–petrol (4:1) gave *Z*-3-methylpent-2-en-1,5-dioic acid (**4**, 166 mg), mp 146–149° (lit. [6] 147–149°). (Found: C, 49.9; H, 5.3, calcd for $C_8H_8O_4$: C, 50.0; H, 5.6%). IR ν_{max} cm^{-1}: 3300, 1714, 1685, 1642. δ_H2.01 (3H, *s*), 3.62 (2H, *s*), 5.89 (1H, *s*); δ_C 26.4 (Me), 39.6 (CH_2), 120.6 ($CH=$), 153.6 ($C=C$), 169.9, 174.5 (both CO_2H).

Rechromatography of the combined oily frs from several fermentations on silica gel and elution with EtOAc–petrol (1 : 3) gave diplodialide B (**3**) as an oil. MS *m/z* 184 $[M]^+$, IR ν_{max} cm^{-1}: 3500, 1720, 1260, 1160, 968. Identified by its ^{1}H and ^{13}C NMR spectra (Table 1). The 3,5-dinitrobenzoate, prepd with 3,5-dinitrobenzoyl chloride in pyridine, had mp 127–128°. (Found: C, 54.1; H, 4.91; N, 7.6. $C_{17}H_{18}N_2O_8$ requires C, 54.0; H, 4.8; N, 7.4%), IR ν_{max} cm^{-1}: 3090, 1735, 1725, 1630, 1600.

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