

## CLADOSPORIN, A NEW ANTIFUNGAL METABOLITE FROM *CLADOSPORIUM CLADOSPORIOIDES*

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(Received for publication August 9, 1971)

Cladosporin is a new fungal metabolite produced in good yield in the mycelium of *Cladosporium cladosporioides* (FRES.) DE VRIES. Its structure was established as 3,4-dihydro-6,8-dihydroxy-3-(tetrahydro-6-methyl-2H-pyran-2-yl)methylisocoumarin on the basis of spectroscopic studies of the parent compound, mono- and di-methyl derivatives, and a monoacetate. Both cladosporin and monoacetyl cladosporin completely inhibited growth of several dermatophytes on agar medium at a concentration of 75  $\mu\text{g/ml}$ . The germination of spores from several *Penicillium* and *Aspergillus* spp. was inhibited by these compounds at concentrations of 40  $\mu\text{g/ml}$  or less in liquid medium.

*Cladosporium cladosporioides* (FRES.) DE VRIES is a field fungus and a common contaminant of many types of seeds. The strain used in the present investigation was isolated from a sample of feed suspected of causing toxicosis and loss of 3,000 broiler chickens in Western Canada. When cultured on a yeast extract-sucrose medium for 7 days the fungus produced large quantities (up to 0.9 g/liter) of a new crystalline phenolic metabolite, designated cladosporin. The metabolite was present in the mycelium only and was readily extracted with hot chloroform. Subsequent analysis of the chicken feed by a tested extraction procedure and thin-layer chromatography (tlc) failed to reveal the presence of cladosporin.

There are no previous reports on production of metabolites by *C. cladosporioides*.

### Experimental

Thin-layer chromatography (tlc) was used to monitor reactions and column chromatography; layers of Merck silica gel (0.25 mm) were developed with toluene-ethyl acetate-90% formic acid (6:3:1) and examined under short wavelength ultraviolet light. Infrared absorption spectra were recorded with a Perkin-Elmer 257 spectrophotometer and ultraviolet absorption spectra with a Cary 14 recording spectrophotometer. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian HR-220 spectrometer; chemical shifts ( $\delta$ ) were measured in ppm downfield from  $\text{Si}(\text{CH}_3)_4$ . Mass spectra were recorded on Hitachi-Perkin Elmer RMU-6 or RMS-4 instruments at 70 or 80 e.v.

#### Production of cladosporin (I)

Twenty 2.8-liter Fernbach flasks, each containing 200 ml yeast extract (2%)-sucrose (15%) liquid medium, were inoculated with spore suspensions of *C. cladosporioides* (from

a monospore culture) and incubated for 7 days at room temperature. The mycelium from each culture was heated in 200 ml chloroform at 60°C for 5 minutes, then soaked overnight in chloroform. Although concentration of the chloroform extracts yielded crystalline cladosporin, it was preferably purified by column chromatography. The concentrated extract (290 ml) from 19 flasks was added to a 312 mm × 29.5 mm (i.d.) column of 86 g Merck silica gel (0.05~0.2 mm particle size). After the column had been washed with 450 ml chloroform, 75 ml fractions containing cladosporin were eluted with 1% acetone in chloroform (fractions 1~3), 2% acetone in chloroform (fractions 4~8), and 5% acetone in chloroform (fractions 9~15). Fractions 6~12 contained most of the cladosporin (2.7 g). Recrystallization from benzene or aqueous ethanol gave colorless needles: m.p. 188.5~189°C;  $[\alpha]_D^{25}$  -24.8° (c 0.96, ethanol);  $\nu_{\max}$  (CHCl<sub>3</sub>) 3575, 3200 (br), 1665, 1630 cm<sup>-1</sup>;  $\lambda_{\max}$  (95% EtOH) 217, 230 (infl.), 270, 303 nm ( $\epsilon$  20,900, 11,950, 13,030, 5,984);  $\lambda_{\max}$  (95% EtOH-NaOH) 242, 311 nm ( $\epsilon$  12,000, 21,000); nmr (CD<sub>3</sub>COCD<sub>3</sub>)-see Fig. 1; mass spectrum (Fig. 2), M<sup>+</sup> 292.

*Anal.* Calcd. for C<sub>16</sub>H<sub>20</sub>O<sub>5</sub>: C 65.75, H 6.90.

Found: C 65.73, H 6.70. No nitrogen was present.

Cladosporin formed a light blue fluorescent spot at Rf 0.55 after tlc. It was soluble in ethanol, and ethyl acetate, moderately soluble in chloroform, slightly soluble in benzene, and insoluble in hexane and water. Cladosporin was soluble in 3 N NaOH but insoluble in 0.1 M NaHCO<sub>3</sub>. GIBBS reagent (1% 2,6-dichloroquinone-4-chloroimide in ethanol, then ammonia) gave a blue color. Methanolic FeCl<sub>3</sub> gave a pale purple color changing to yellow-brown with excess reagent; no reaction product was detected by tlc.

#### Monomethyl cladosporin (II)

A mixture of 159 mg cladosporin, anhydrous K<sub>2</sub>CO<sub>3</sub> (58 mg), methyl iodide (1 ml), and acetone (6 ml) was refluxed for 46 hours with occasional addition of more methyl iodide and acetone. The solution was filtered, evaporated, and the residue purified by column chromatography on silica gel; benzene-chloroform (1:1) eluted monomethyl cladosporin which was recrystallized from methanol-water as colorless plates (94 mg): m.p. 98~98.5°C;  $\nu_{\max}$  (CHCl<sub>3</sub>) 3100 (br), 1665, 1628 cm<sup>-1</sup>;  $\lambda_{\max}$  (95% EtOH) 216.5, 227 (infl), 267, 302 nm ( $\epsilon$  21,900, 12,510, 13,480, 5,535);  $\lambda_{\max}$  (95% EtOH-NaOH) 231, 237, 268, 335 nm ( $\epsilon$  26,400, 26,100, 8,690, 7,770); nmr (CDCl<sub>3</sub>)-see Fig. 3; mass spectrum (Fig. 2), M<sup>+</sup> 306 (m/e 40, 44, and 58 are contaminants).

*Anal.* Calcd. for C<sub>17</sub>H<sub>22</sub>O<sub>5</sub>: C 66.65, H 7.24.

Found: C 66.86, H 7.47.

Like cladosporin, the monomethyl ether gave a positive GIBBS test and FeCl<sub>3</sub> color and was soluble in 3 N NaOH. It also formed a light blue fluorescent spot on tlc, at Rf 0.80. The compound was generally more soluble in organic solvents than cladosporin.

#### Dimethyl cladosporin (III)

Cladosporin (141.5 mg) was heated at 80°C for 20 minutes with dimethyl sulfate (1 ml) and 3 N NaOH, added as required to maintain alkalinity. The acidified reaction mixture was extracted with 4 × 3 ml ethyl acetate. The extracts were dried, filtered, and evaporated, then purified by column chromatography on silica gel (elution with 1% acetone in chloroform, after initial chloroform wash) followed by preparative tlc on a 0.8-mm layer of silica gel with development by benzene-acetic acid (9:1). The major deep blue fluorescent band was removed and eluted with chloroform-methanol (1:1). Recrystallization from isopropyl ether gave colorless needles of dimethyl cladosporin: m.p. 120~120.5°C;  $\nu_{\max}$  (CHCl<sub>3</sub>) 1709, 1605 cm<sup>-1</sup>;  $\lambda_{\max}$  (95% EtOH) 216, 225 (infl), 263, 298 nm ( $\epsilon$  24,593, 6,716, 13,483, 6,285)-unchanged on addition of NaOH-EtOH; mass spectrum (Fig. 2), M<sup>+</sup> 320 (m/e 149, 205, and 223 are background contaminants).

*Anal.* Calcd. for C<sub>18</sub>H<sub>24</sub>O<sub>5</sub>: C 67.48, H 7.55.

Found: C 67.60, H 7.71.

Dimethyl cladosporin was insoluble in 3 N NaOH. It gave a deep blue fluorescent spot (Rf 0.37) on tlc.

#### Monoacetyl cladosporin (IV)

Cladosporin (146.5 mg) was heated on the steam bath for 4 hours with acetic anhydride (5 ml). Evaporation of the acetic anhydride yielded a crystalline residue, which was purified by elution with benzene-chloroform (1:1) from a silica gel column and recrystallized from *n*-hexane as colorless plates: m.p. 127°C;  $\nu_{\max}$  (CHCl<sub>3</sub>) 3120 (br), 1765, 1670, 1620 cm<sup>-1</sup>;  $\lambda_{\max}$  (95 % EtOH) 212, 252, 307 nm ( $\epsilon$  28,956, 8,928, 4,815);  $\lambda_{\max}$  (95 % EtOH-NaOH) 244, 312 nm ( $\epsilon$  10,867, 20,330); nmr (CD<sub>3</sub>COCD<sub>3</sub>)  $\delta$  11.30 (1 H, singlet), 6.65 (1 H, doublet,  $J=2$  Hz), 6.61 (1 H, multiplet), 4.82 (1 H, multiplet), 4.08 (1 H, multiplet), 3.88 (1 H, multiplet), 3.08 (2 H, AB part of ABX system,  $J_{AB}=16$  Hz), 2.28 (3 H, singlet), 2.15 (1 H, multiplet), 1.79 (1 H, multiplet), 1.67 (4 H, multiplet), 1.32 (2 H, 8 line multiplet), 1.13 (3 H, doublet,  $J=6.5$  Hz); mass spectrum 334 (M<sup>+</sup>), 316, 292, 274, 256, 236, 194, 179, 178, 176, 151, 150, 125, 99, 81, 69, 66.3 (metastable), 55, 43.

*Anal.* Calcd. for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>: C 64.66, H 6.63.

Found: C 64.77, H 6.75.

Monoacetyl cladosporin formed a light blue fluorescent spot on tlc at Rf 0.79.

Acetylation of cladosporin with acetic anhydride and pyridine at room temperature gave, in addition to a small amount of the monoacetate, a gummy solid after separation by preparative tlc. The material formed a spot on tlc at Rf 0.53 visualized by its deep blue fluorescence after spraying with conc. H<sub>2</sub>SO<sub>4</sub>. It could not be obtained crystalline but appeared to be diacetyl cladosporin:  $\nu_{\max}$  (CHCl<sub>3</sub>) 1770, 1720, 1615 cm<sup>-1</sup>; mass spectrum, 376 (M<sup>+</sup>), 334, 333, 99, 81.

#### Microbiological methods

(1) Media: For studies on the inhibition of spore germination by cladosporin and derivatives the liquid germination medium (LGM) (pH 6.6) described by BRIAN and HEMMING (1945)<sup>11</sup> was employed; for growth of the microorganisms and assay of the compounds the following media were employed: tryptic soy agar (TSA) (Difco) for bacteria and yeast; Sabouraud dextrose agar (SDA) (Difco) and potato dextrose agar prepared with fresh potatoes (FPDA), according to the Difco formula, for dermatophytes and fungal plant pathogens.

(2) Compounds assayed: Solutions of cladosporin, monomethyl cladosporin, and monoacetyl cladosporin in acetone were added to the various media (acetone concentration  $\leq 4$  %). Because of the low solubility of these compounds in water, we generally restricted their highest final concentrations in the agar test media to 75  $\mu$ g/ml and in LGM to 40  $\mu$ g/ml.

(3) Spore germination method: Spore crops harvested from 7 days old culture slopes of *Aspergillus* and *Penicillium* species were suspended in LGM containing 0.025 % Tween 80 and diluted with LGM to obtain  $2.5 \times 10^8$  spores per ml. An improved Neubauer hemacytometer was used for counting the spores. 0.5 ml Cups in clear Linbro "disposito trays" (Winley Morris Diagnostics, Montreal, Canada) were loaded with 0.1 ml of each spore suspension and 0.1 ml LGM containing the test compounds (for final concentrations, see Table 1). Tests and acetone controls were run in duplicate. The sterilized trays, lightly covered with plastic film to avoid evaporation, were incubated at 25°C, and 200 spores/cup were examined for germination with an inverted microscope at 16 and 24 hours. A spore was defined as germinated when the germ tube length exceeded half the minor diameter of the spore.

(4) Agar dilution method: The concentrations of the compounds incorporated in the agar media after autoclaving are listed in Table 2. Control media contained only the acetone solvent. A loopful of each TS broth suspension prepared from 24 hours slope cultures of bacteria and yeast were spotted on duplicate test and control plates. Inhibition of growth was recorded after 24 hours incubation at 37°C. Tufts of mycelium of

dermatophytes (10 days old cultures), and plant pathogens (7 days old cultures) were point inoculated on the appropriate media and incubated at 25°C for 6 days. On the 6th day complete inhibition of growth was verified with an inverted microscope.

## Results and Discussion

### Structure of Cladosporin

The molecular formula of cladosporin was established as  $C_{16}H_{20}O_5$  by elemental analysis and a parent ion at  $m/e$  292 in the mass spectrum. This was confirmed by preparation of a monomethyl derivative  $C_{17}H_{22}O_5$ , a dimethyl derivative  $C_{18}H_{24}O_5$ , and a monoacetate  $C_{18}H_{22}O_6$ . Peaks in the ultraviolet absorption spectra of cladosporin and the monomethyl derivative closely resembled those reported<sup>2-4</sup> for 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin and 3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin in both wavelength and extinction coefficients. The spectra of both cladosporin and monomethyl cladosporin changed on addition of alkali, as expected for phenols, while the dimethyl derivative was unchanged. In monoacetyl cladosporin, the 217 and 270 nm peaks of cladosporin were now at 212 and 252 nm, indicating the acetate was aromatic, and we obtained tlc evidence in addition to spectral indications that addition of alkali caused hydrolysis back to cladosporin.

The 3,4-dihydro-6,8-dihydroxyisocoumarin nucleus for cladosporin was supported by its infrared spectrum, which showed bands at 3575 and 3200  $cm^{-1}$  corresponding to free and hydrogen-bonded hydroxyl groups, respectively, and a hydrogen-bonded lactone band at 1665  $cm^{-1}$ . The 3575  $cm^{-1}$  band was absent in the infrared spectra of the monomethyl and monoacetyl derivatives and the 1665  $cm^{-1}$  band was not shifted. Hydrogen bonding was removed in the dimethyl derivative, which had no hydroxyl bands, and the lactone band now appeared at 1709  $cm^{-1}$ .

Additional evidence for the 3,4-dihydro-6,8-dihydroxyisocoumarin part of the cladosporin molecule was obtained from the nmr spectra of cladosporin and its monomethyl and monoacetyl derivatives. The nmr spectra of cladosporin (Fig. 1), monomethyl cladosporin (Fig. 3), and monoacetyl cladosporin had signals at  $\delta$  11.30, 11.15, and 11.30 p.p.m. respectively, corresponding to a phenolic proton hydrogen-bonded to the *peri*-carbonyl group. The second phenolic proton appeared as a broad band at

Fig. 1. 220 MHz nuclear magnetic resonance (nmr) spectrum of cladosporin (in  $CD_3COCD_3$ ).

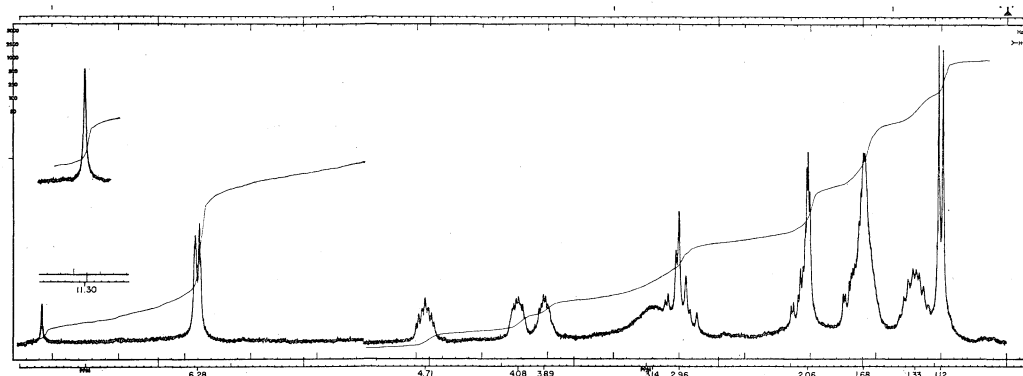
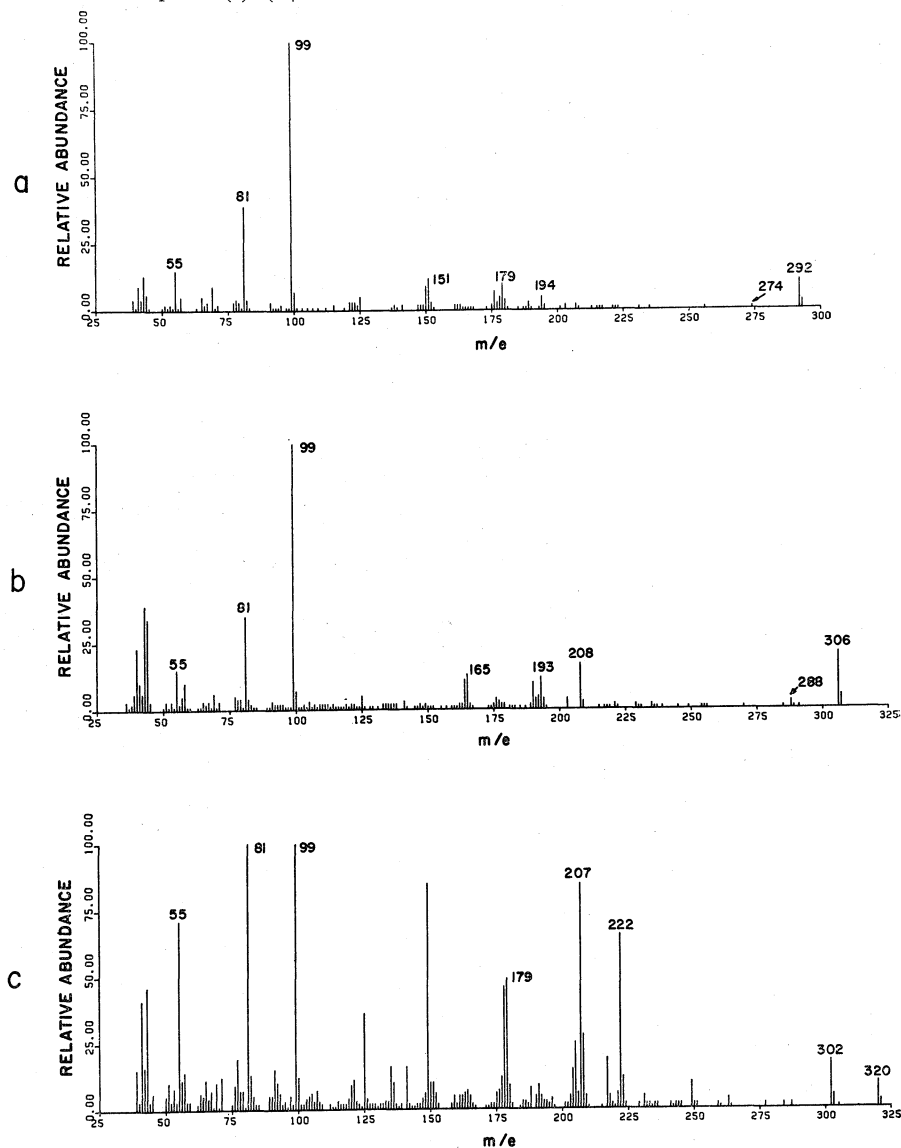
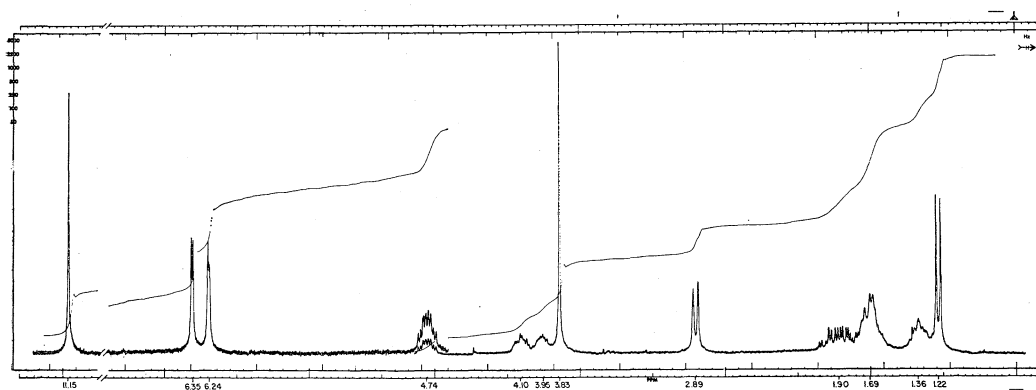


Fig. 2. Mass spectra of cladosporin (a), monomethyl cladosporin (b), and dimethyl cladosporin (c) (m/e 149 is attributable to di-*n*-butyl phthalate background).

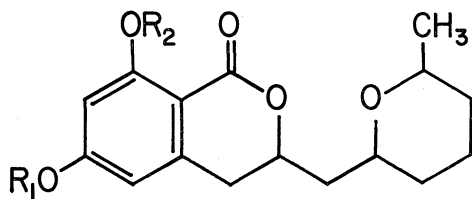


3.14 p.p.m. in the spectrum of cladosporin, which was absent in the derivatives; the band moved downfield with addition of 1 drop  $D_2O$  and coalesced with the HDO signal at 3.9 p.p.m. after addition of 2 more drops  $D_2O$ . Although at high field for a phenolic proton, such chemical shifts are not unknown, *cf.* reticulol possesses a phenolic hydroxyl signal at 3.40 p.p.m. in deuterated dimethylsulfoxide.<sup>5)</sup> The aromatic region of the nmr spectrum of cladosporin (Fig. 1) contains split signals for two protons at about 6.28 p.p.m.; the coupling constant ( $J=2$  Hz) is more clearly measurable in the nmr spectrum of the monomethyl derivative (Fig. 3) and is typical of *meta*-coupled aromatic protons. A multiplet (AB part of ABX system,  $J_{AB}=16$  Hz) at 2.96 p.p.m. (2 protons) in the cladosporin spectrum and 3.08 p.p.m. in the mono-

Fig. 3. 220 MHz nmr spectrum of monomethyl cladosporin (in  $\text{CDCl}_3$ ).

acetate is assigned to a benzylic  $\text{CH}_2$  group; this group appears at 2.89 p.p.m. as a doublet in the spectrum of monomethyl cladosporin (Fig. 3). The multiplet at 4.71 p.p.m. (1 proton) in cladosporin is due to the adjacent  $\text{CH}(\text{O}\cdot\text{CO})$  proton at position 3. Irradiation of this peak affects the 2.96 p.p.m. multiplet (in addition to other signals), and irradiation of the corresponding 4.74 p.p.m. multiplet in monomethyl cladosporin reduces the doublet at 2.89 p.p.m. to an apparent singlet; conversely irradiation at 2.89 p.p.m. affects the 4.74 p.p.m. multiplet. Thus cladosporin is a 3,4-dihydro-6,8-dihydroxyisocoumarin substituted in the 3 position by a  $\text{C}_7\text{H}_{13}\text{O}$  group.

The side chain at position 3 of the dihydroisocoumarin system contains two  $-\text{CHO}-$  groups appearing as separate 1 proton multiplets at 4.08 and 3.89 p.p.m. in the nmr spectrum of cladosporin and at very similar positions in the spectra of the monomethyl and monoacetyl derivatives. Clearly these groups share the remaining oxygen atom, and as they do not correspond to epoxide methine protons, they must be part of a ring. Irradiation at 3.89 p.p.m. (3.95 p.p.m. in the monomethyl derivative) collapses the  $\text{CH}_3$  doublet at 1.12 p.p.m. (1.22 in the methyl derivative) (3 protons,  $J=6.5$  Hz) to a singlet; conversely (in the monomethyl derivative), irradiation at 1.22 p.p.m. affects the 3.95 p.p.m. multiplet. The presence of a  $\text{CH}_3\text{CHOCH}$  group is thus established. The nature of the cyclic moiety containing this group was apparent from the mass spectra of cladosporin and its derivatives (Fig. 2). Each mass spectrum contains common peaks at  $m/e$  125, 99, 81, 69, 55, and 43 with a metastable peak at  $m/e$  66.3 corresponding to loss of 18 mass units from  $m/e$  99. The latter peak is due to a tetrahydromethylpyranlyl group in which the methyl group must be in the 6 position. This leads to structure I for cladosporin. The analogous loss of water



- I,  $\text{R}_1=\text{R}_2=\text{H}$
- II,  $\text{R}_1=\text{CH}_3$ ;  $\text{R}_2=\text{H}$
- III,  $\text{R}_1=\text{R}_2=\text{CH}_3$
- IV,  $\text{R}_1=\text{COCH}_3$ ;  $\text{R}_2=\text{H}$

from the P-1 peak in the mass spectrum of tetrahydropyran has been discussed by COLLIN and CONDE-CAPRACE<sup>6</sup>;  $m/e$  99 (base peak) and 81 are also prominent ions in the mass spectrum of 2-(2-methyl-2-propenyl)-4-methyltetrahydropyran,<sup>7</sup> which is a useful model for cladosporin.

Apart from prominent parent ions, the mass spectra (Fig. 2) of cladosporin (I), monomethyl cladosporin (II), and dimethyl cladosporin (III) also show ions attributable to loss of water (P-18), loss of tetrahydro-6-methylpyranyl with acquisition of one proton (P-98), loss of methyl from P-98 (P-113), and loss of tetrahydro-6-methylpyran-2-ylacetaldehyde (with and without acquisition of an extra proton) (P-141, P-142). The latter two processes are analogous to the loss of  $\text{CH}_3\text{CO}\cdot$  and acetaldehyde from 3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin<sup>8</sup> and the corresponding 7-chloro compound.<sup>4</sup>

Assignments of nmr chemical shifts to the remaining protons were made on the basis of decoupling experiments with cladosporin (I) and monomethyl cladosporin (II). The two protons in the methylene bridge appear as a multiplet at 1.90 p.p.m. in the monomethyl derivative; irradiation of the adjacent  $\text{CH}(\text{O})$  signal at 4.74 p.p.m. reduced this multiplet to the AB part of an ABX system and irradiation of the other adjacent CH peak at 4.10 p.p.m. also collapsed the multiplet. The methylene bridge in cladosporin itself appears at 2.11 p.p.m. and 1.76 p.p.m. Irradiation of either signal, at 4.10 p.p.m. and 3.95 p.p.m., due to the two  $\text{CH}(\text{O})$  protons in the methyltetrahydropyran ring of monomethyl cladosporin, affects peaks at both 1.36 and 1.69 p.p.m., in addition to changes referred to earlier. Thus each ring  $\text{CH}_2$  group adjacent to these  $\text{CH}(\text{O})$  groups gives rise to signals at both 1.36 and 1.69 p.p.m. The remaining ring  $\text{CH}_2$  group must have a chemical shift of 1.69 p.p.m. also. Similar assignments were deduced for the ring  $\text{CH}_2$  protons in cladosporin giving signals at 1.33 and 1.68 p.p.m.

Cladosporin is an addition to the growing list of 3,4-dihydroisocoumarins produced by fungi, although 3,4-dihydro-6,8-dihydroxyisocoumarins are comparatively rare. 3,4-Dihydro-6,8-dihydroxy-3,4,5-trimethylisocoumarin, (+)-3,4-dihydro-6,8-dihydroxy-3,4,5-trimethylisocoumarin-7-carboxylic acid, and (+)-3,4-dihydro-3,4,5,7-tetramethyl-3,6,8-trihydroxyisocoumarin are metabolites of *Penicillium citrinum* structurally related to the antibiotic citrinin.<sup>9</sup> (-)-3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin has been isolated from an *Aspergillus terreus* mutant<sup>2</sup> and its 6-methyl ether from stored carrots<sup>3</sup> and from *Sporormia* spp.<sup>4</sup> and *Penicillium gladioli*.<sup>8</sup> The latter compound is known to be fungistatic to certain species.<sup>10</sup> 3,4-Dihydro-6,8-dihydroxy-3- $[\beta$ -(4-methoxyphenyl)ethyl]isocoumarin has been isolated from the roots of the plant *Agrimonia pilosa*.<sup>11</sup> Only one compound containing the unsubstituted tetrahydro-6-methylpyran-2-yl moiety appears to have been previously isolated from fungi; averufanin<sup>12</sup>, which has the structure 1,3,6,8-tetrahydroxy-2-(tetrahydro-6-methyl-2H-pyran-2-yl)anthraquinone, is a metabolite of *Aspergillus versicolor*, *A. flavus*, and *Bipolaris* sp. The mass spectrum of averufanin does not show ions of  $m/e$  99 and 81, because formation of a fragment of  $m/e$  99 would involve the unlikely cleavage of a carbon-carbon bond next to a double bond system, a point discussed

by BIEMANN<sup>7)</sup> with regard to 2-(2-methyl-2-propenyl)-4-methyltetrahydropyran and its 1-propenyl isomer.

### Antibiotic Activity of Cladosporin and Derivatives

Cladosporin at a concentration of 10~20  $\mu\text{g/ml}$  in LGM inhibited germination of 50% or more of the spores from 7 of the 12 strains of *Penicillium* and *Aspergillus*

Table 1. Percent inhibition of spore germination, based on percent germination of control, by different concentrations of cladosporin and monoacetyl cladosporin ( $\mu\text{g/ml}$ )

Organism	Cladosporin										Monoacetyl cladosporin									
	16 hours					24 hours					16 hours					24 hours				
	40	20	10	5	2.5	40	20	10	5	2.5	40	20	10	5	2.5	40	20	10	5	2.5
<i>Penicillium viridicatum</i> 69-22	100	100	100	85	85	95	65	65	<50		89	78	73	73	73	85	55	<50		
<i>Aspergillus niger</i> 141404 U	100	95	85	65	<50	95	85	70	70	<50	100	85	70	<50		78	56	<50		
<i>P. viridicatum</i> 69-23	100	90	85	70	<50	100	95	55	<50		100	100	100	96	60	100	100	80	<50	
<i>P. rubrum</i> F3	100	95	80	60	60	85	85	75	<50		93	88	76	76	76	—*	—	—	—	—
<i>A. ochraceus</i> 66-87	99	97	77	61	<50	75	<50				85	75	<50			<50				
<i>A. versicolor</i> IMI 49124	95	70	55	<50		95	85	85	85	75	84	67	67	<50		60	60	60	<50	
<i>P. expansum</i> H71	85	85	75	55	<50	60	60	<50			100	100	96	96	96	96	92	92	92	80
<i>A. flavus</i> V71	85	65	60	60	<50	75	<50				84	67	<50			<50				
<i>A. versicolor</i> IMI 16134	85	<50				<50					68	52	<50			<50				
<i>P. viridicatum</i> W70-63	75	65	<50			<50					100	100	84	50	<50	78	56	<50		
<i>A. clavatus</i> W70-33	75	<50				75	65	55	<50		100	97	84	84	<50	100	98	90	70	60
<i>A. nidulans</i> W70-119	55	<50				<50					<50					<50				

\* —: not done

Table 2. Effect of cladosporin and its monomethyl and monoacetyl derivatives on dermatophytes, plant pathogens, bacteria, and yeast

Organism	Medium	Days	M. I. C. ( $\mu\text{g/ml}$ )		
			Cladosporin	Monomethyl cladosporin	Monoacetyl cladosporin
<i>Trichophyton interdigitale</i>	SDA, FPDA	6	75	>75	75
<i>T. rubrum</i>	"	"	75	>75	75
<i>T. tonsurans</i>	"	"	75	75	75
<i>Microsporon canis</i>	"	"	75	>75	>75
<i>Epidermophyton floccosum</i>	"	"	75	>75	75
<i>Botrytis cinerea</i>	"	"	>75	>75	>75
<i>Rhizoctonia solani</i>	"	"	75	>75	>75
<i>Stemphylium radicinum</i>	"	"	>75	>75	>75
<i>Escherichia coli</i>	TSA	1	>75	>75	>75
<i>Bacillus subtilis</i>	"	"	>75	>75	>75
<i>Bacillus cereus</i>	"	"	>75	>75	>75
<i>Staphylococcus aureus</i>	"	"	>75	>75	>75
<i>Staphylococcus epidermidis</i>	"	"	>75	>75	>75
<i>Micrococcus flavus</i>	"	"	40	>75	75
<i>Saccharomyces cerevisiae</i>	"	"	>75	75	>75



tested after 24 hours (Table 1). Spores of *A. niger* were swollen an average of 37% more than controls in the presence of 75  $\mu\text{g/ml}$  cladosporin and, where germination occurred, tubes were wide and distorted. Of the various microorganisms against which cladosporin was tested, *Micrococcus flavus* appeared to be the most sensitive (Table 2). At 75  $\mu\text{g/ml}$  cladosporin inhibited the growth of five dermatophytes, belonging to three different genera, and the plant pathogen *Rhizoctonia solani*.

The activity of monoacetyl cladosporin closely paralleled that of cladosporin (Tables 1 and 2), and while it did not inhibit the growth of *Microsporon canis* or *R. solani*, it did restrict the growth of these organisms to approximately one quarter and one twentieth, respectively, of the diameters of the corresponding control cultures. Monomethyl cladosporin was the least active of the 3 compounds tested, and inhibited germination of 50% of the spores from only 3 of the 12 fungi tested at a concentration of 40  $\mu\text{g/ml}$ . However, it alone inhibited the growth of *Saccharomyces cerevisiae* (Table 2).

#### Acknowledgements

We are grateful to B. KENNEDY and D. ANYETI for technical assistance, A. A. GREY of the Ontario Research Foundation for the 220 MHz nmr spectra and valuable advice, G. F. MORRIS, Canada Department of Agriculture for the elemental microanalyses, J. A. VON ARX, Centraalbureau voor Schimmelcultures-Baarn, for identification of *C. cladosporioides*, C. RIDDELL, University of Saskatchewan, for the chicken feed, and L. KAPICA, McGill University, for the dermatophytes.

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