CULPIN, A NOVEL HYDROQUINONE ANTIBIOTIC OF FUNGAL ORIGIN

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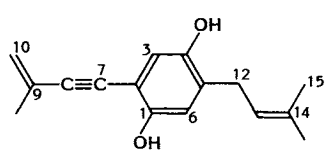
Culpin, **1**, is a novel antibiotic found during the course of screening for new antifungal agents. It is produced by a species of *Preussia* isolated from a soil sample collected in Culpeper, Virginia. In this note, we describe the taxonomy of the producing organism and the production, isolation, physicochemical properties and structure elucidation of this novel antibiotic.

The producing organism is a saprophytic, coprophilous ascomycete that readily produces sexual fruiting bodies bearing asci and ascospores when grown on potato-glucose agar or tomato juice agar. Histological examination of thin sections shows the fruiting body to be a cavity within stromatic tissue. The asci are arranged in parallel fascicles arising from the base of the cavity. Paraphyses (long filiform cells) are interspersed between the asci. The asci are eight-spored, broadly clavate, with a basal stem attached to a crozier, the site where meiotic division occurs. There is no pore at the apex of the ascus or any other means of dehiscence. At maturity, the ascus wall becomes evanescent, and the spores are liberated. The ascospores are $8.2 \times 5.4\,\mu m$, dark brown, opaque, thick-walled and have an elongated germinal slit extending the full length of each cell. Some spores with transverse septa divide mitotically, doubling the number of ascospores per ascus. At maturity, ascospores within an ascus may number as many as thirty two. These characteristics conform with those given by CAIN[1] for members of the genus, *Preussia*. The culture has been deposited in the American Type Culture Collection with the accession No. ATCC 20923.



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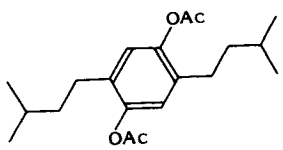
Production of the antibiotic was accomplished by growing the organism in a medium consisting of toasted Nutrisoy flour 1.5%, soluble starch 1.5%, glucose 5%, $CoCl_2 \cdot 6H_2O$ 0.0005% and $CaCO_3$ 1% for 120 hours on a rotary shaker at 25°C. This growth was then used to inoculate flasks containing the following medium: Malt extract 1%, yeast extract 1%, peptone 0.1% and glucose 2%. Maximum antibiotic production was obtained after 120 hours incubation at 25°C on a rotary shaker. Production and subsequent isolation steps were followed by paper disc-agar diffusion assay with *Saccharomyces cerevisiae*, and by TLC (Merck Silica gel 60, $CHCl_3$-EtOAc, 3:7, Rf 0.71). The antibiotic is readily isolated by MeOH extraction of the mycelial cake, subsequent silica gel chromatography with $CHCl_3$-MeOH (98:2) and recrystallization from benzene-heptane to give culpin as off-white crystals, mp 98~100°C. Two liters of whole broth gave 360 g (wet weight) of mycelium which yielded 92.8 mg of culpin. (The same fermentation also produces preussin[2], a compound with the same gross structure and relative stereochemistry as L-657,398[3]). Culpin is detected by fluorescence quenching, I_2, phosphomolybdic acid, Pauly reagent and $FeCl_3$, but gives no response with ninhydrin or Rydon-Smith reagents. The IR spectrum ($CHCl_3$) shows strong bands at 3590, 3515 (OH stretch), 2960, 2905 (CH), aromatic ring stretch bands at 1600, 1485 and 1435, and a weak band at 2180 cm^{-1} indicative of a internal alkyne. The UV spectrum (MeOH) has maxima (ε) at 210 (30,000), 252 (13,400), 265 (16,500), 280 (17,400) and 326 nm (11,800). The spectrum is unchanged in acid, but an irreversible change is observed in 0.01 N NaOH in MeOH to give maxima (ε) at 206 (30,000), 278 (13,200) and 451 nm (4,300). The ^1H and ^{13}C NMR spectra, Table 1, show signals for 18 protons (two exchangeable) and 16 carbons, respectively. This data, combined with a high-resolution fast atom bombardment mass spectrum (HRFAB-MS) ($(M)^+$ *m/z* 242.1287, calcd 242.1302) led to a molecular formula of $C_{16}H_{18}O_2$.

Acetylation (CH_2Cl_2, 4-dimethylaminopyridine, acetic anhydride) of culpin produced a diacetate as a white solid. Hydrogenation of the diacetate (H_2, 10% Pd-C, 1 atm) gave a product with greatly simplified ^1H and ^{13}C NMR spectra, Table 1. The reduction had obviously produced a symmetrical aromatic diacetate with two isopentyl units as substituents. Since the ^1H NMR spectrum of the natural product contains resonances for two

Table 1. ^1H and ^{13}C NMR data for culpin (**1**), **2** and quinone (**5**).

Carbon No.	1		2		5	
	^1H[a]	^{13}C[b]	^1H[b]	^{13}C[b]	^1H[b]	^{13}C[b]
1, 4	4.17 (1H, s, OH)	150.5 (s)		146.4 (s)		188.0 (s)
	5.39 (1H, s, OH)	147.3 (s)				
2		107.3 (s)		133.2 (s)		149.6 (s)
3, 6	6.57 (1H, s)	121.0 (d)	6.88 (2H, s)	123.4 (d)	6.53 (2H, t[c])	132.5 (d)
	6.85 (1H, s)	117.5 (d)				
5		134.9 (s)		133.2 (s)		149.6 (s)
7		97.0 (s)	2.45 (4H, m)	27.7 (t)	2.40 (4H, m)	26.5 (t)
8		82.1 (s)	1.43 (4H, m)	39.0 (t)	1.38 (4H, m)	36.9 (t)
9		126.2 (s)	1.56 (2H, m)	27.9 (d)	1.60 (2H, m)	27.9 (d)
10	5.27 (1H)[c]	122.5 (t)	0.91 (12H, d)	22.4 (q)	0.93 (12H, d)	22.3 (q)
	5.02 (1H)[c]					
11	1.71 (3H)[c]	23.4[d] (q)				
12	3.19 (2H, d)	29.5 (t)				
13	5.22 (1H, t)[c]	115.6 (d)				
14		130.8 (s)				
15	1.47 (3H, br s)	17.8 (q)				
16	1.54 (3H, br s)	25.7[d] (q)				
CO				169.4 (s)		
$COCH_3$			2.29 (6H, s)	20.8 (q)		

[a] In C_6D_6; chemical shifts, δ, are in ppm downfield from TMS.
[b] In $CDCl_3$; chemical shifts, δ, are in ppm downfield from TMS. Carbon chemical shift assignments are based on empirical correlations.
[c] Multiplet due to small couplings, $J=1$ to 2 Hz.
[d] Assignments may be interchanged.



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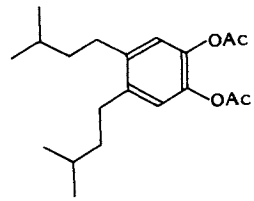

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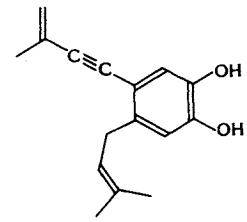

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Table 2. Activity of culpin *in vitro*.

Organism	MIC (μg/ml)[a]
Staphylococcus aureus FDA 209 P	>100
Streptococcus agalactiae SC9287[b]	50
Micrococcus luteus SC2495	100
Mycobacterium fortuitum SC8571	50
Escherichia coli SC10909	100
Candida albicans SC5314	100
C. tropicalis SC2963	50
C. krusei SC2969	100
C. glabrata SC9342	100

[a] MIC by agar dilution assay.
[b] SC is the Squibb culture collection designation.

aromatic protons that are singlets (hence *para* to each other) only two structures, **2** and **3**, are consistent with the NMR data for the reduced product. Thus, culpin must have structure **1** or **4**. These two possibilities were differentiated by oxidation of the reduced, non-acylated antibiotic with ceric ammonium nitrate[4] and examination of the ^{13}C NMR spectrum of the resulting quinone (**5**). The carbonyl carbons are found at δ 188.0 which is typical of a *para* (δ 187.4~188.2), but not an *ortho* (δ 179.2~180.9), alkyl quinone[5]. Culpin therefore is a novel hydroquinone antibiotic, 2-(3-methyl-2-butenyl)-5-(3-methyl-3-buten-1-ynyl)-1,4-benzenediol, **1**.

Culpin has weak antimicrobial activity against a variety of bacteria and fungi whose MIC values are listed in Table 2.

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