(*all trans*)-2,4,6,8,10,12-Tetradecahexene-1,14-dial, a New Pigment from *Conidiobolus paulus*

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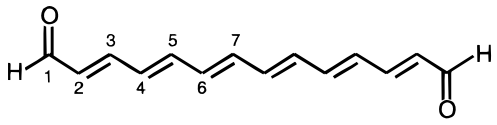
A study of the coloring matter produced by *Conidiobolus paulus* Drechsler NRRL 2648 on potato-dextrose medium led to the isolation of a new dialdehyde unsaturated metabolite, (*all trans*)-2,4,6,8,10,12-tetradeca-1,14-hexenedial. The structure was characterized by MS and by ^{1}H- and ^{13}C-NMR. The compound inhibited the growth of the Gram-positive bacterium, *Bacillus cereus*, and the Gram-negative bacterium, *Escherichia coli* (MIC values of 10 μg and 50 μg, respectively).

The fungal genus *Conidiobolus* is morphologically complex. Although most of its members are saprophytic, some can exist as parasites on animals and insects. The best known member of this genus is *C. coronatus*, a fungus that infects termites and aphids and is also a human pathogen.[1] A literature search revealed that fatty acids are the main metabolites characterized among species of *Conidiobolus*. Arachidonic acid has been shown to be produced by *Conidiobolus* spp. in small amounts, which indicates some potential for intracellular lipid production.[2] Fatty acids elaborated by species of *Conidiobolus* have been studied extensively by Tyrell *et al.*[3,4] as a chemotaxonomic aid for their classification. For example, a distinctive feature of the lipids produced by *C. denaesporus* is the high percentages of myristic and eicosatetraenoic acids.[5] Recently, Kendrick and Ratledge[6,7] reported the production of short-chain polyunsaturated fatty acids by *C. denaesporus*.

This report describes a new natural product, unsaturated dialdehyde pigment **1**, which was isolated from mycelia of *C. paulus* NRRL 2648. Previously, the pigment had been prepared synthetically.[8,9] The present study was undertaken as part of a survey for potentially novel pigmented substances from fungi. The antibacterial activity of the pigment was evaluated by an agar disk-diffusion assay.



Extraction with Me_2CO of mycelia grown 13 days on potato-dextrose (PD) broth gave crude pigment. This pigment was crystallized as fine red needles from DMF, mp 215−217 °C. Elemental analysis and HRMS were in agreement for the molecular formula $C_{14}H_{14}O_2$. The IR spectrum (KBr pellet) of **1** indicated a carbonyl group (1650 cm^{-1}) conjugated to double bonds and a lower frequency band (1590 cm^{-1}) for conjugated double bonds. Because **1** exhibited only limited solubility in most organic solvents, its ^{1}H- and ^{13}C-NMR spectra were determined in deuteriopyridine (C_5D_5N). The ^{1}H-NMR spectrum of **1** revealed a proton at δ 9.70 for a carbon bearing an oxygen and that all double bonds were trans. The ^{13}C-NMR showed shifts for **1** in $C_5D_5N_5$ at δ 193.3 for a carbonyl carbon and 12 olefinic carbons between δ 132.0 and 151.4, with one peak in this region obscured by pyridine. The assignments were further confirmed by a ^{1}H−^{1}H COSY NMR experiment. Thus, compound **1** was assigned as (*all trans*)-2,4,6,8,10,12-tetradecahexene-1,14-dial.

The potential antibacterial activity and toxicity towards chick embryo were evaluated as described elsewhere.[10,11] No toxicity was noted in the chick embryo assay at levels up to 100 μg (data not shown). Antibiotic activity by contact was evident for the Gram-positive *Bacillus cereus* NRRL B-3705 and the Gran-negative *Escherichia coli* B-3704 at minimum concentrations of 10 μg and 50 μg, respectively. This is the first report of a pigment elaborated by a species *Conidiobolus* and is a novel compound from a biological source.

Experimental Section

General Experimental Procedures. Melting points (uncor) were done with a Fisher-Johns apparatus, IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and UV-vis measurements were taken with a Beckman DU spectrophotometer. EIMS were obtained at 70 eV using the probe method with a Nuclide 90 spectrometer. The NMR spectra were recorded on a 300 MHz Bruker WM-300 spectrometer.

Fungal Maintenance. *Conidiobolus paulus* was obtained from the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research (NCAUR) (1815 North University Street Peoria, IL 61604), as NRRL 2648. The organism was maintained on PD agar. Sterile PD liquid medium (250 mL) in 1000-mL Erlenmyer flasks with cotton plugs was inoculated with 1 mL of a 5-mL suspension of a 7-day-old growth of *C. paulus* from PD agar slants. The flasks containing the inoculated liquid medium were incubated at 25 °C with reciprocal shaking (95 cycles/min with a 5-cm throw) for 13 days. The 13-day PD fermentation medium was replated on tryptone-glucose-yeast extract (TGY), trypticase-soy, and PD agar media, and there was no evidence for bacterial or fungal contamination after 72 h. The fermented liquid broth (1 L) was centrifuged (10 000 rpm) for 10 min. Suspended cells

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were separated and washed (4 ×) with distilled H_2O. The washed cells were extracted with Me_2CO (500 mL) for 2 h at room temperature, and the Me_2CO extract was then separated from the cells by filtration and dried over Na_2SO_4. After filtration from the drying agent, the solvent was evaporated to leave a red oil (405 mg).

Isolation of 1. The red oil was triturated with hexane, and the remaining red solid collected by filtration was dried *in vacuo* to yield 207 mg of crude pigment. The crude pigment was crystallized from DMF to give the major component **1** as red needles (151 mg, mp 215−217 °C, uncor); IR (KBr) ν max 1650, 1590, 1135, 1100, 1010 cm^{-1}; UV (CS_2) λ max (log ϵ) 350 (sh, 47,047), 386 (sh, 78,953), 404 (90,850), 424 (sh, 80,035) nm; ^1H NMR (C_5D_5N, 300 MHz) δ 9.70 (1 H, d, C(O)−H, J = 7.9 Hz, H-1), 6.28 (1 H, dd, CH=CH, J = 15.2 Hz, H-2,3), 7.19 (1 H, dd, CH=CH, J = 11.5 Hz, H-3,4), 6.54 (1 H, m, CH=CH, J = 15.7 Hz, H-4,5), 6.77 (1 H, m, CH=CH, J = 11.5 Hz, H-5,6), 6.56 (1 H, m, CH=CH, J = 15 Hz, H-6,7), 6.59 (1 H, m, CH=CH, J = 15 Hz, H-7,8); ^{13}C NMR (C_5D_5N, 75.4 MHz) δ 193.28 (C-1, C-14), 151.44 (d, C-3, C-12), 142.28 (d, C-7−C-8), 138.14 (d, C-4−C-11), 132.14 (d, C-5−C-12), 13214 (d, C-5−C-12) and one carbon obscured by C_5D_5N at 135.17 (d, C-6−C-9); EIMS (70 eV) m/z [M^+] 214 (65), [M − CHO], 185 (8), 167 (10), 146 (15), 129 (35), 115 (50), 91 (100), 65 (35), 81 (40), 79 (48), 77 (60), 39 (40); HREIMS m/z 214.0989 ($C_{14}H_{14}O_2$ requires 214.0993). *Anal.* Calcd for $C_{14}H_{14}O_2$: C, 78.05; H, 6.76. Found: C, 78.48; H, 6.58.

Antibiotic Disk Assay. Slant cultures of *B. cereus* B-3705 and *E. coli* B-3704 obtained from the Agricultural Research Service Culture Collection (NCAUR, 1815 North University Street, Peoria, IL 61604) were each maintained on TGY agar. The bacteria were incubated 24 h at 32 °C before assay plates were poured. Cell suspensions of each bacterial species were prepared by dilution in TGY broth to give an optical density (1 cm) of 0.15 at 600 nm. The standardized inoculum (0.1 mL) was added to 5 mL of melted TGY agar cooled to 45 °C. Inoculated agar was poured into a 100 × 15 mm petri dish. Compound **1** at concentrations of 1, 5, 10, 25, 50, and 100 μg per assay disk was placed on the agar plates. Inhibitions by contact were visible after 24 h when compared to disks that received solvent only. MICs of compound **1** were 10 and 50 μg/assay disk (repeated 3 ×) against *B. cereus* and *E. coli*, respectively.

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