# PHLOROGLUCINOL DERIVATIVES FROM *AEROMONAS HYDROPHILA*

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(Received for publication August 7, 1978)

A bacterial isolate from decayed roots of a greenhouse-grown red pine seedling, *Pinus resinosa*, when cultured on malt agar or potato dextrose agar (PDA) showed inhibitory activity against selected test fungi. Sensitive fungi included *Ceratocystis ulmi* (BUISM.) C. MOREAU, *Pythium* spp., *Rhizoctonia solani* KUHN, *Cylindrocarpon* sp., *Fusarium oxysporum* SCHLECT., and *Sirococcus strobilinus* PREUSS.

When grown on PDA, a colorless crystalline solid was observed in association with the bacterial colony. With a view to studying further the fungitoxic principle(s), the bacterium, subsequently identified as *Aeromonas hydrophila* subsp. *anaerogenes**, was grown at 24°C in submerged shake culture in Erlenmeyer flasks each containing 500 ml of sterile potato broth. The latter medium was prepared from 200 g cubes $(1 \sim 2\text{cm})^3$ of potato, *Solanum tuberosum*, var. KENNEBEC.

The cultures were extracted after various time intervals by stirring vigorously with two successive 250 ml volumes of ether. Bioassay of the solvent-free extracts indicated the presence of fungitoxic material. Optimal production of ether-soluble metabolites was attained in 2~3 days. Thus, a two-day 500-ml culture of the bacterium yielded, from the ether extracts 234 mg of brown semi-solid material. TLC analysis (silica gel GF 254, type 60, E. Merck; toluene - acetone, 4: 1) revealed three major spots, corresponding to metabolites designated A, B, and C, with Rf *ca.* 0.6, 0.4, and 0.2, respectively. Preparative thin-layer chromatography afforded A (88 mg), B (84 mg), and C (10 mg), all as pale yellow solids which were rendered almost colorless following sublimation or recrystallization. Red-brown, violet, and yellow colorations, respectively, were observed on treatment of A, B, and C in methanol with ferric chloride solution.

Metabolite C (sublimed at 160~180°C/0.1Torr), mp 207~215°C, was identified as phloroglucinol by comparison of spectra and tlc behavior with an authentic sample, and by mixture melting point. Mol. wt. calcd. for $C_6H_6O_3$: 126; found (ms): 126.

Similarly, compound B (crystallized from methanol - water), mp 219~223°C, was identified as 2′,4′,6′-trihydroxy acetophenone (phloracetophenone), on the basis of its spectra and by direct comparison and mixture melting point with an authentic sample (Aldrich Chemical Co. Inc.). Mol. wt. calcd. for $C_8H_8O_4$: 168.0422; found (hrms): 168.0420. Base peak: *m/e* 153.0184 $(M-CH_3)^+$.

The least polar of the three metabolites, A, crystallized from methanol - water as needles, mp 168~172°C; ir (KBr) (*inter alia*) 3650~2100, 1645~1575, 1435, 1405, 1365, 1295, 1230, and 1200 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 270 nm ($\varepsilon$ 26,200); $\lambda_{max}$ (alkaline MeOH) 288 nm ($\varepsilon$ 26,800)[3]; pmr ($CD_3OD$) $\delta$ 2.63 (s, 6H), 5.76 (s, 1H) (hydroxyls exchanged); hrms *m/e* 210.0531 (calcd. for $C_{10}H_{10}O_5$: 210.0528), 195.0293 (base peak) $(M-CH_3)^+$, 177.0187$(M-CH_3$ and $H_2O)^+$. On the basis of these data, A was tentatively identified as 2,4-diacetylphloroglucinol, a conclusion corroborated by direct comparison and mixture melting point with an authentic sample, prepared according to CAMPBELL and COPPINGER[3].

Disc sensitivity tests with purified A, B, and C at 100 and 50 $\mu$g/disc indicated that the modest antifungal activity of the ether extract resided in metabolite A, 2,4-diacetylphloroglucinol. Pro-

* The organism has been identified according to BERGEY's Manual[1] but differs from the classical description in certain respects. It is VOGUES PROSKAUER negative and therefore can be categorized as a biotype 2. In addition, growth was exhibited at pH 10.0, the range being 5.0~10.0, and the temperature range for growth was extended to 45°C. This organism does not utilize maltose or trehalose and indole is not produced from tryptophan. With the mentioned exceptions, this organism fits the accepted description. A much broader test base was utilized and has been described in studies on numerical taxonomy of isolates from natural environments.[2]

duction of the latter, together with phloracetophenone and 2,4,6-triacetylphloroglucinol, by a bacterium, *Pseudomonas fluorescens*, has been reported previously by REDDI *et al.*[4~6]. In assays conducted by these authors, 2,4-diacetylphloroglucinol at 100 or 1,000 $\mu$g/ml exhibited high antibiotic activity against Gram-positive bacteria and actinomycetes, but had little or no effect on Gram-negative bacteria, fungi or yeasts[6].

Acknowledgements

The technical assistance of Messrs B. ROZE and J. R. CORMIER in part of this study is acknowledged. Thanks are due to Mr. A. I. BUDD, University of Alberta, Edmonton, for the accurate mass measurements.

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