PHLOROGLUCINOL DERIVATIVES
FROM AEROMONAS HYDROPHILA

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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 in-
cluded Ceratocystis ulmi (BUISM.) C. MOREAU,
Pythium spp., Rhizoctonia solani KUHN, Cylin-
drocarpon sp., Fusarium oxysporum SCHLECT.,
and Sirococcus strobilinus PREUSS.

When grown on PDA, a colorless crystalline
solid was observed in association with the bac-
terial colony. With a view to studying further
the fungitoxic principle(s), the bacterium, subse-
quently identified as Aeromonas hydrophila subsp.
aerogenes*, 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 (1~2cm)³
of potato, Solanum tuberosum, var. KENNEBEC.
The cultures were extracted after various time
intervals by stirring vigorously with two suc-
cessive 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, cor-
responding 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 color-
less following sublimation or recrystallization.
Red-brown, violet, and yellow colorations, re-
spectively, 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 C₆H₆O₃: 126; found
(ms): 126.

Similarly, compound B (crystallized from
methanol - water), mp 219~223°C, was identi-
fied as 2',4',6'-trihydroxy acetophenone (phlor-
acetophenone), 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 C₈H₈O₄: 168.0422; found
(hrms): 168.0420. Base peak: m/e 153.0184
(M-CH₃)+.

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 λmax (MeOH) 270 nm (e 26,200);
λmax (alkaline MeOH) 288 nm (e 26,800)³; pmr
(CD₂OD) δ 2.63 (s, 6H), 5.76 (s, 1H) (hydroxyls
exchanged); hrms m/e 210.0531 (calcd. for C₁₀
H₁₀O₅: 210.0531), 195.0293 (base peak) (M-
CH₃)+, 177.0187(M-CH₃ and H₂O)+. On the
basis of these data, A was tentatively identified
as 2,4-diacetylphloroglucinol, a conclusion cor-
roborated by direct comparison and mixture
melting point with an authentic sample, prepared
according to CAMPBELL and COPPINGER³).

Disc sensitivity tests with purified A, B, and C
at 100 and 50 µg/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 Manual³ 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 ex-
ceptions, 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)

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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 µg/ml exhibited high antibiotic activity against Gram-positive bacteria and actinomycetes, but had little or no effect on Gram-negative bacteria, fungi or yeasts6).

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References