

C-ACETYLPHLOROGLUCINOLS FROM *PSEUDOMONAS FLUORESCENS*

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(Received 11 June 1976)

Key Word Index—*Pseudomonas fluorescens*; bacteria; phloracetophenone; 2,4-diacetylphloroglucinol; 2,4,6-triacetylphloroglucinol.

The bacterium *Pseudomonas fluorescens* was isolated from a soil sample by R. Baker working in these laboratories (No. 5499 in our collection, now deposited with NCIB as No. 11241). When grown on nutrient broth supplemented with glucose the medium was found to possess antibacterial properties.

The antibiotic mainly responsible for this activity is 2,4-diacetylphloroglucinol (identical with a synthetic sample [1]) which is effective at a concentration of 10 µg/ml against the following organisms: *Streptococcus pyogenes*, *S. faecalis*, *S. mutans*, *S. sanguis*, *Staphylococcus aureus*, *Clostridium welchii*, *Lactobacillus casei*. A second metabolite, not antibacterial at concentrations below 100 µg/ml, was identified by comparison with synthetic material as phloracetophenone [2]. A third metabolite was detected on TLC by virtue of its antibacterial properties, but was not obtained in the crystalline state. This ran faster than 2,4-diacetylphloroglucinol on the TLC plates (silica gel: CHCl₃-MeOH, 24:1) and the mass spectrum of a concentrate showed a parent ion at *m/e* 252.0633, which is the mass of 2,4,6-triacetylphloroglucinol. This was synthesised [2] and was found to have the same mobility on TLC and the same antibacterial properties.

Although all three metabolites are known compounds, this is the first time that any of them have been isolated as natural products.

EXPERIMENTAL

Production and isolation of the metabolites. *Pseudomonas fluorescens* (NCIB 11241, No. 5499 in our collection) was

grown under stirred aerated conditions on a medium containing (g/l): Lab Lemco paste (10) bacteriological peptone (10), NaCl (5) glucose (5). After 20 hr at 25° the broth was centrifuged at 4300 rpm for 15 min and the supernatant (6l; natural pH) was extracted with EtOAc (4 × 1200 ml) and the extract (2.52 g) chromatographed over a column of Si gel (270 g) eluting initially with CHCl₃ (B.P. grade) and later with CHCl₃ containing small amounts of MeOH. An early fraction gave an amorphous brown solid (15 mg) with antibacterial activity against *C. welchii*, parent ion in mass spec. at *m/e* 252.0633 (C₁₂H₁₂O₆ requires 252.0634). Two crystalline compounds were obtained from later fractions. 2,4-Diacetylphloroglucinol (530 mg), crystals from aqueous ethanol mp 168–170° (lit. [2] 168°); cherry red colour with FeCl₃ in EtOH; λ_{max} (EtOH), 206 nm (ε 18 500), 269 nm (ε 5200); ν_{max} 3500, 1630, 1310 cm⁻¹; found: C, 54.8; H, 5.2. Calc. for C₁₆H₁₀O₅·½H₂O: C, 54.8; H, 5.0%. Phloracetophenone (984 mg), crystals from aqueous ethanol mp 213–220° (lit. [1] 217–218°); violet colour with FeCl₃ in EtOH; λ_{max} (EtOH), 228 nm (ε 14 500), 288 nm (ε 16 200); ν_{max} 3600, 3500, 1645, 1290, 1170, 1070 cm⁻¹; τ 4.15 (ArH), 7.41 (CH₃CO). For comparison, synthetic samples of phloracetophenone, 2,4-diacetylphloroglucinol and 2,4,6-triacetylphloroglucinol were prepared according to the published procedures.

Acknowledgements—We thank Dr. T. D. Hennessey and his staff for full antibacterial testing and Mr. A. B. Davies for fermentation work.

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DEHYDROZINGERONE FROM *AFRAMOMUM GIGANTEUM*

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(Received 8 June 1976)

Key Word Index—*Aframomum giganteum*; Zingiberaceae; phenolic compounds; anthraquinone; dehydrozingerone; syringaldehyde; syringic acid; emodin.

Plant. *Aframomum giganteum* K. Schum (syn. *Amomum giganteum* Oliv. and Hanb, Zingiberaceae). *Source.* The stems were collected in Central Africa Republic by Mr. R. Pujol and Mr. P. Teocchi of the Experimental Station of La Maboké. They were identified by Professor R.

Tomaselli, Institute of Botany (Pavia). *Previous work.* Kaempferol 3,7,4'-trimethyl ether, quercetin 3,7,4'-trimethyl ether (ayanin), quercetin 3,7,3',4'-tetramethyl ether (retusine), chrysophanol, physcion, 2,6-dimethoxybenzoquinone and sitosterol have been isolated [1].