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C-ACETYLPHLOROGLUCINOLS FROM *PSEUDOMONAS FLUORESCENS*

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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. faecaelis, 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: $CHCl_3$–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 $CHCl_3$ (B.P. grade) and later with $CHCl_3$ 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 ($C_{12}H_{12}O_6$ 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 $FeCl_3$ in EtOH; λ_{max} (EtOH), 206 nm (ε 18500), 269 nm (ε 5200); ν_{max} 3500, 1630, 1310 cm^{-1}; found: C, 54.8; H, 5.2. Calc. for $C_{10}H_{10}O_5\frac{1}{2}H_2O$: C, 54.8; H, 5.0%. Phloracetophenone (984 mg), crystals from aqueous ethanol mp 213-220° (lit. [1] 217–218°); violet colour with $FeCl_3$ in EtOH; λ_{max} (EtOH), 228 nm (ε 14500), 288 nm (ε 16200); ν_{max} 3600, 3500, 1645, 1290, 1170, 1070 cm^{-1}; τ 4.15 (Ar*H*), 7.41 ($C\underline{H}_3CO$). For comparison, synthetic samples of phloracetophenone, 2,4-diacetylphloroglucinol and 2,4,6-triacetylphloroglucinol were prepared according to the published procedures.

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DEHYDROZINGERONE FROM *AFRAMOMUM GIGANTEUM*

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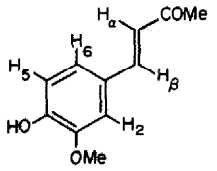
Key Word Index—*Aframomum giganteum*; Zingiberaceae; phenolic compounds; anthraquinone; dehydrozingerone; syringaldehyde; syringic acid; emodin.

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-dimenthoxybenzoquinone and sitosterol have been isolated [1].

Present work. Further study on the $CHCl_3$ extract of the stems has now yielded emodin, syringaldehyde, syringic acid and dehydrozingerone (**1**). The structures of the first three compounds were determined by spectroscopic data and by comparison with authentic samples. Dehydrozingerone has been never found before in nature, although it was already known as synthetic product.



Scheme 1.

The compounds were separated on a polyamide column and purified on Si gel columns. (**1**), mp 120–124°, showed in the IR spectrum (KBr) bands at ν_{max} 3300 cm^{-1} (OH), 1670 (α,β-unsaturated C=O), 1640 and 970 cm^{-1} (*t*-CH=CH-). The PMR spectrum (60 MHz, $CDCl_3$, TMS) showed the following functional groups: MeCO δ 2.35 (3H, *s*); CH_3O δ 3.92 (3H, *s*); *trans* HC=CH δ 6.60 (1H, *d*, $J_{\alpha\beta}$ 16.0 Hz, H_α) and δ 7.48 (1H, *d*, $J_{\alpha\beta}$ 16.0 Hz, H_β); 1,2,4-trisubstituted benzene δ 6.90 (1H, *d*, J_{5-6} 9.5 Hz, H-5) and δ 7.0–7.2 (2H, *m*, H-2 and H-6). λ_{max}^{EtOH} nm (log ϵ): 240 (3.93), 337 (4.29), which by addition of MeCOONa were shifted to 257 and 406 nm, indicating a free OH group in 4-position. MS (probe, 70 eV, *m/e* rel. int.): 192 M^+ (100); 177 (M^+-15, 95); 145 (71). The structure was finally confirmed by synthesis [2]: condensation of vanillin and acetone in presence of 10% NaOH followed by purification on a Si gel column afforded the 4(4'-hydroxy-3'-methoxyphenyl)3-buten-2-one, mp 128–129° identical with the natural compound in all respects.

It is interesting that some compounds related to (**1**) have been found in other Zingiberaceae: zingerone, gingerols, shogaols and paradols in *Zingiber officinale* Roscoe [3, 4] and in *Amomum melegueta* Roscoe [5, 6]. Dehydrozingerone could arise biogenetically by the condensation of 4-hydroxy-3-methoxycinnamic acid with one malonate unit, followed by double decarboxylation.

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1-(3-GLUCOSYLOXY-4-HYDROXYCINNAMYL)GLUCOSE FROM *LANTANA HYBRIDA*

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The presence of 1-caffeylrhamnose in the flowers of *Lantana hybrida* has been reported recently [1]. A new compound (R_f 0.22 in *n*-BuOH–HOAc–H_2O, 4:1:5) was isolated from extracts of the above plant material (collected in Catania, Italy) by preparative PC. The UV spectrum (λ_{max} 335 nm in EtOH) showed a large bathochromic shift (75 nm) in the presence of NaOEt. Controlled acid hydrolysis (10% HOAc; 3.5 hr under reflux) gave D-glucose identified by PC and by the use of glucose oxidase. Methylation with MeI–AgO in $HCONME_2$ [2] followed by acid hydrolysis (0.3 N HCl; 4 hr under reflux) gave 2,3,4,6-tetra-*O*-methyl-D-glucose identified by PC and TLC. Acid hydrolysis (1 N HCl; 0.5 hr at 100°) and treatment with β-glucosidase gave D-glucose and caffeic acid in the ratio of 2.07:1, identified by PC and TLC. Since the compound gave no colour reaction with sodium molybdate [1], the *o*-diphenolic group of caffeic acid is not free. Methylation with CH_2N_2 followed by acid hydrolysis (2 N HCl; 0.5 hr at 100°) gave isoferulic acid identified by PC [3] and TLC; hence the 4-hydroxyl of caffeic acid is free and the 3-hydroxyl is bound by a β-linkage to D-glucose. Since alkaline hydrolysis (0.2 N NaOH; 3 hr at room temp) and treatment with esterase [4] gave D-glucose, this sugar is attached from its 1-position by a β-linkage to the carboxyl group of caffeic acid. The mass spectrum of the permethyl ether showed a parent ion at *m/e* 630 (calc. MW 630). Thus the compound must be 1-[3-(β-D-glucosyloxy),4-hydroxycinnamyl]glucose, which has not previously been reported in plants.

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