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DANIELONE, A PHYTOALEXIN FROM PAPAYA FRUIT

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Key Word Index—*Carica papaya*; Caricaceae; *Colletotrichum gloesporioides*; phytoalexin induction; acetophenone; antimicrobial activity.

Abstract—A new phytoalexin was induced and isolated from papaya fruit slices treated with copper salts; its structure was established as 3′,5′-dimethoxy-4′-hydroxy-(2-hydroxy)acetophenone. This compound showed high antifungal activity against *Colletotrichum gloesporioides*, a pathogenic fungus of papaya. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Phytoalexins are antimicrobial compounds produced by plants when they are exposed to microorganisms [1]; however, the same type of compounds are isolated when plants are challenged to abiotic conditions, such as UV radiation, freezing or heavy-metal (mainly copper) salts [2, 3]. Papaya (*Carica papaya*) is an edible fruit that is very susceptible to the fungal pathogen *Colletotrichum gloesporioides*, which causes rapid rotting 1–2 days after infection. This fungus is also pathogenic to tomato tree fruit and causes the disease known as anthracnosis. We have isolated other classes of phytoalexins from this plant [4, 5]. This paper describes the induction and the characterization of a new phytoalexin, named danielone **1**, from papaya fruit. This compound has a high antifungal activity against *Colletotrichum*.

RESULTS AND DISCUSSION

Danielone (**1**) was isolated as a powder; the high-resolution EI-mass spectrum showed the $[M]^+$ at *m/z* 212.20, in agreement with a molecular formula $C_{10}H_{12}O_5$. The presence of hydroxyl, cationic carbonyl and an aromatic ring was indicated by the IR absorptions at 3450, 1670, 820, 840 and 780 cm^{-1}. The ^1H NMR spectrum (Table 1) shows two singlets at δ 3.95 (6 H) and δ 7.19 (2 H) ppm, that were attributed to methoxyl groups and aromatic protons, respectively; compound **1** also displayed two signals at δ 6.14 and 3.57 ppm interchangeable with D_2O addition. The latter was coupled with a doublet at δ 4.83 ppm, as deduced from a ^1H–^1H COSY experiment. In the ^{13}C NMR spectrum the occurrence of a carbonyl function was supported by the resonance at δ 196.65 and the DEPT experiment displayed four quaternary carbons, one methine, one methylene, and one methyl group. The possibility of a symmetric molecule was obvious to satisfy the formula obtained from high resolution EI-mass spectrometry.

The structure of danielone (**1**) was deduced using a combination of HMQC and HMBC experiments. For instance, the carbonyl group displayed ^3J correlation with H-6′ and H-2′ protons (δ 7.19 in ^1H NMR and 147.08 ppm in ^{13}C NMR) and ^2J correlation with protons of a methylene group at δ 4.83 (Fig. 1), while H-2′ and C-6′ are mutually coupled through long-range correlation and both signals showed ^2J correlation with a quaternary at 124.79 ppm (C-1′) and with a quaternary and oxygenated carbon at 140.74 ppm (C-4′). Other important couplings are depicted in Fig. 1. However, the relative position of the two methoxyl groups and the hydroxyl group was established only by means of X-ray diffraction analysis of its 2,4′-diacetate derivative (Fig. 2). Danielone (**1**) inhibited the germination of spores of *C. gloesporioides* at concentrations

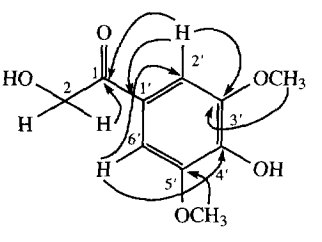

Fig. 1. Structure of danielone (**1**) and correlations obtained with a HMBC experiment.

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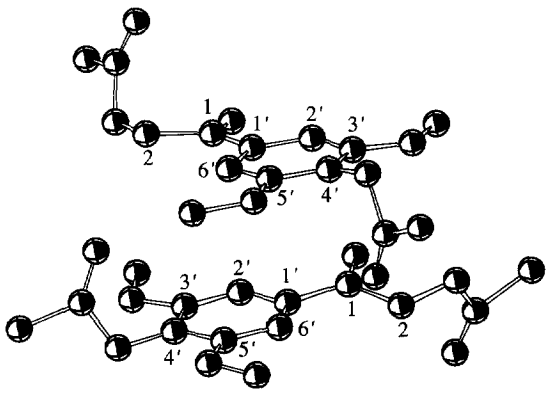

Fig. 2. ORTEP view of danielone.

of approximately 50–75 ppm. Several acetophenones have been reported as phytoalexins or constitutive antifungal agents from species of the Rosaceae [6].

EXPERIMENTAL

General. MS (HR): VG-Micromass ZAB-2F at 70 eV; MS (LR): HP5995 at 70 eV. The ^1H and ^{13}C spectra were obtained on a Bruker AMX400 (100 MHz) and WP200SY (50 MHz), using $CDCl_3$ and TMS as standard int. ref. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Standard pulse sequences were used for COSY, DEPT, HMQC and HMBC experiments. TLC was developed on precoated silica gel G-25 (Merck); CC on Kieselgel 60 (70–230 mesh, Merck). Visualization of the TLC plates was achieved with oleum spray reagent.

Isolation and detection. Papaya fruit (35 kg) were peeled, cut in 1.5-cm thick slices and soaked for 48 hr in $CuCl_2$ or $CuSO_4$ soln (0.1 M) with Tween 20 (0.5%). Slices were then ground in 20 l of EtOH (95%) in a blender, centrifuged and filtered through cheesecloth. The filtrate was concentrated *in vacuo* to give a dark-brown gum (1.4 g) which was re-extracted with *n*-hexane, CH_2Cl_2 and EtOAc. Sterile H_2O/Tween was used as a control treatment. Compound **1** was detected only in the EtOAc extracts from fruit treated with $CuCl_2$ or $CuSO_4$, by comparison of their composition on TLC using $CHCl_3$–Me_2CO (9:1). Danielone (50 mg) was purified from dark gum by CC in the fr. eluted with *n*-hexane–CH_2Cl_2 (9:1). Danielone: amorphous solid, mp 145°, UV λ_{max}^{MeOH} 227, 298 nm. MS: 70 eV, $[M]^+$, 212.20, $C_{10}H_{12}O_5$ (22%), 181 $[M-OCH3]^+$ (100%), 153(12), 123(10), 95(8), 67(13).

X-ray crystallographic analysis of $C_{14}H_{16}O_7$, triclinic, space group P1 $a = 11.153(16)$, $b = 12.944(15)$, $c = 12.707(15)$Å, $V = 1491.2(12)$Å^3 and $z = 4$. Data were measured in a Siemens AED4 diffractometer with Cu-Kα radiation graphite monochromator) using $\omega:\theta$ scans. The structure was solved by direct methods using the SHELXS-86 program. Anisotropic temperature factors were used for the refinement of the non-H atoms. The final discrepancy index was $R = 0.013$. The crystallographic data have been deposited at the Cambridge Crystallographic Centre.

Antimicrobial assay. Colletotrichum gloesporioides was originally obtained from diseased fruits of *C. papaya*. Compound **1** was tested as ethanolic solutions (5%) to 50, 75 and 100 ppm and antifungal activity was established by counting the germination of spores at days 3, 8 and 15 after inoculation. Each compound was tested in triplicate and a control experiment containing only solvents was conducted.

Table 1. ^1H and ^{13}C NMR data for **1*** (in $CDCl_3$)

Position	^1H	^{13}C	HMBC
1		196.65 *s*	7.19, 4.83
2	4.83, *d*, *J* = 1.9 Hz	64.95 *t*	
1′		124.79 *s*	7.19, 4.83
2′	7.19 *s*	104.98 *d*	7.19
3′		147.08 *s*	3.96, 7.19
4′		140.74 *s*	7.19
5′		147.08 *s*	3.96, 7.19
6′	7.19 *s*	104.98 *d*	7.19
3′-OCH3	3.95 *s*	56.54 *q*	
5′-OCH3	3.95 *s*	56.54 *q*	
2-OH	6.14 *s*		
4′-OH	3.14, *J* = 1.9 Hz		

*Assignments were made with ^1H–^1H COSY, DEPT and HMQC.

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