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A 2-ARYLBENZOFURAN PHYTOALEXIN FROM COWPEA (*VIGNA UNGUICULATA*)

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Key Word Index—*Vigna unguiculata*; Leguminosae; cowpea; 2-arylbenzofuran; *Colletotrichum lindemuthianum*; phytoalexins.

Abstract—2-(2′-methoxy-4′-hydroxyphenyl)-6-methoxybenzofuran (vignafuran) has been identified as the major phytoalexin from cowpea leaves infected with *Colletotrichum lindemuthianum*.

INTRODUCTION

In recent years considerable attention has been paid to the antifungal compounds (phytoalexins) produced in response to fungal or virus infection by species of the Leguminosae, notably by French Bean, *Phaseolus vulgaris* L. These compounds have been shown to be isoflavanoids comprising pterocarpans, isoflavans and the isoflavanone kievitone [1–3]. An investigation of the phytoalexins of cowpea, *Vigna unguiculata* (L.) Walp., infected with *Colletotrichum lindemuthianum* has revealed the presence of phytoalexins of these classes [4], but in addition an antifungal 2-arylbenzofuran has been isolated, to which structure (**1**) is assigned. This compound has been named vignafuran.

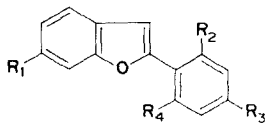
RESULTS AND DISCUSSION

Vignafuran was isolated from the leaves of cowpea seedlings grown in the light, following inoculation with *Colletotrichum lindemuthianum*. In these circumstances it is the major phytoalexin present in the leaves, the yield being of the order of 30 mg/kg fr. wt. It is present in similar abundance in the leaves of infected seedlings grown in the dark. Only trace quantities were found in the stems of light-grown infected seedlings and the compound was not detected in the etiolated stems of dark-grown infected plants, nor in uninfected control plants.

Vignafuran gives a dark yellow colour in conc H_2SO_4 and a purple–brown colour on TLC when sprayed with diazotized *p*-nitroaniline. The UV spectrum is in accord with a 2-arylbenzofuran structure [5,6], showing absorption maxima of 320 and 335 nm, which shift in alkali to 336 and 347 nm respectively. The MS is simple, with the molecular ion at *m/e* 270 and a peak at *m/e* 135 corresponding to the doubly-charged molecular ion. Also prominent are peaks at *m/e* 255 (M-15), *m/e* 227 (M-15-28) and *m/e* 212 (M-15-28-15). The fragmentation pathway linking these peaks is established by the presence of metastable ions. Examination of the molecular ion by high resolution MS showed the elemental composition to be $C_{16}H_{14}O_4$. This composition requires two methoxyl groups and one hydroxyl group to be attached to the 2-phenylbenzofuran skeleton and these groups are observed in the NMR spectrum.

Comparison of the NMR spectrum of vignafuran with published data for 2-arylbenzofurans [6–8] identifies the two protons with the lowest chemical shifts as H-6′ and H-4. Both of these protons appear as *ortho* doublets (J 9Hz), showing that C-5 and C-5′ are unsubstituted. The H-6′ and H-4 signals do not show *meta* splitting, implying substitution at C-6, C-2′ and C-4′. On spectroscopic evidence there are thus three possibilities for the arrangement of the substituent groups in vignafuran (**1–3**).

The H-3′ and H-5′ protons of vignafuran appear in the NMR spectrum as a multiplet centred at 3·45 τ. In the spectrum of *O*-acetylvignafuran the signals from these protons again



(1) $R_1 = R_2 = OMe$, $R_3 = OH$, $R_4 = H$

(2) $R_1 = R_3 = OMe$, $R_2 = OH$, $R_4 = H$

(3) $R_1 = OH$, $R_2 = R_3 = OMe$, $R_4 = H$

(4) $R_1 = R_2 = OMe$, $R_3 = H$, $R_4 = OH$

overlap and cannot be distinguished individually, but have undergone a downfield shift relative to vignafuran of *ca* 0·3 τ. This shift is ascribed to loss of the shielding effect of a phenolic hydroxyl group *ortho* or *para* to the H-3′ and H-5′ protons. Furthermore, the chemical shift of the H-5 and H-7 protons is virtually unaffected by acetylation of vignafuran. Structure **3** is therefore eliminated, as it has a hydroxyl group attached at C-6. The choice between **1** and **2** was made on the basis of the Gibbs test [9]. A negative result for vignafuran indicated substitution *para* to the hydroxyl group, inferring the correct structure to be **1**.

Support for the structure was obtained by synthesis. 2-Benzyloxy-4-methoxyphenylacetonitrile reacted with 3-methoxyphenol under the conditions of the Hoesch synthesis to give a mixture of three compounds identified as 2-arylbenzofurans by their UV spectra. Only one of these gave a negative Gibbs test, and this compound had NMR and UV spectra, and R_f, identical with those of vignafuran; a mmp determination on the *O*-acetyl derivatives of synthetic and of naturally-occurring vignafuran showed no depression.

Vignafuran is the most active of the phytoalexins so far isolated from cowpea in tests against isolates of I47 and I57, the two prevalent Nigerian races of *Colletotrichum lindemuthianum*, giving total inhibition of spore germination at 8 ppm.

EXPERIMENTAL

General. Cowpea seed was obtained from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria, who also supplied Nigerian isolates of *Colletotrichum lindemuthianum*. NMR spectra were recorded at 60 MHz. Mps are uncorrected.

Isolation of Vignafuran (**1**). Cowpea seed (cv. New Era, I.I.T.A. accession line TVu 57) was sown in moist vermiculite and grown at 25° under daylight fluorescent lighting for 4 days. After inoculation with *C. lindemuthianum* isolate I47 and incubation at 16° for 7 days, the leaves (740 g) bearing small brown lesions were detached and macerated in MeOH (200 ml). The MeOH extract was filtered, evaporated to dryness and the residue was partitioned between H_2O (100 ml) and Et_2O (5 × 50 ml). After drying over $MgSO_4$ the combined ether phases were evaporated to leave a residue which was applied to a column of silica gel (100 mesh) adjusted to pH 7. Fractions from the column were examined by TLC and those eluted with $CHCl_3$–hexane (3:1) which gave a purple–brown spot with diazotized *p*-nitroaniline were combined, concentrated and purified by TLC on Si gel plates using $CHCl_3$–EtOH (97:3) and hexane–EtOAc (3:1) to give a glassy residue of *vignafuran* (23 mg): λ_{max}^{EtOH} nm (log ϵ) 210 (4·48), 224 (shoulder) (4·20), 282 (4·17), 308 (shoulder) (4·37), 320 (4·59), 335 (4·52). NMR ($CDCl_3$, τ): 2·15 (*d*, *J* 9·3, H-6′), 2·57 (*d*, *J* 9·0, H-4), 2·95 (*m*, H-3, H-7), 3·15 (*q*, J_1 9·0, J_2 2·7, H-5), 3·45 (*m*, H-3′, H-5′), 6·10 (*s*, OMe), 6·15 (*s*, OMe). MS *m/e* (% base peak): 271 (14·0), 270 (100), 269 (8·1), 256 (10·3), 255 (78·5), 227 (9·1), 212 (8·7), 135 (11·0).

O-Acetylvignafuran. Prepared with Ac_2O and pyridine in the usual manner, this compound crystallized as needles from MeOH, mp 94–94·5°. NMR ($CDCl_3$, τ): 1·98 (*d*, *J* 9, H-6′), 2·53 (*d*, *J* 8·3, H-4), 2·72 (*s*, H-3), 2·90–3·21 (*m*, H-5, H-7, H-3′, H-5′), 6·03 (*s*, OMe), 6·13 (*s*, OMe), 7·70 (*s*, OCOMe).

Synthetic vignafuran and isomers (**2**) *and* (**4**). A mixture of 2-benzyloxy-4-methoxyphenylacetonitrile [10] (4·5 g), 3-methoxyphenol (7·1 g), fused $ZnCl_2$ (3·2 g) and $AlCl_3$ (80 mg) in dry ether (8·0 ml) was cooled to 0° and saturated with dry HCl. After 5 days at 0° the ether was decanted from the residual pink solid which was washed 2× with dry Et_2O and heated with H_2O (60 ml) on a steam bath for 4 hr. The products were extracted with Et_2O, which was washed with H_2O and aq. $NaHCO_3$ and dried ($NaSO_4$). TLC silica gel chromatograms of the concentrated ether solution developed in $CHCl_3$–MeOH (19:1) and visualized with diazotized *p*-nitroaniline showed a mixture of isomeric products, which was separated by preparative TLC (Si gel, $CHCl_3$–MeOH, 19:1) to yield *vignafuran* (45 mg), identical in spectroscopic and chromatographic properties with the naturally-occurring compound.

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