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2′-*O*-METHYLPHASEOLLIDINISOFLAVAN FROM INFECTED TISSUE OF *VIGNA UNGUICULATA*

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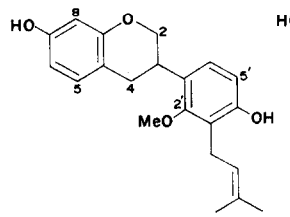
Key Word Index—*Vigna unguiculata*; Leguminosae; cowpea; 2′-*O*-methylphaseollidinisoflavan; *Colletotrichum lindemuthianum*; phytoalexins.

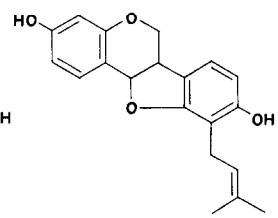
Plant. Cowpea, *Vigna unguiculata* (L.) Walp., accession lines TVu 32, 37, 57, 317, 400, 2276, 2366, 2398. *Source*: International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria. *Previous work*: antifungal compounds [1].

Present work: At least seven antifungal compounds (phytoalexins) are produced by cowpea (cv. New Era, I.I.T.A. accession line TVu 57) following inoculation with *Colletotrichum lindemuthianum*. The structure of one of the principal phytoalexins from infected stems is reported here. This compound gives an orange colour with diazotized *p*-nitroaniline and, from its UV spectrum, belongs to the *iso*-flavanoid class of phytoalexin commonly encountered in the Leguminosae. The 60 MHz NMR spectrum reveals it to be an *iso*flavan unsubstituted at C-6′, deduced from the chemical shifts of the heterocyclic ring protons [2]. Thus the protons at C-2 appear as a quartet at 5·7 τ and a triplet at 6·07 τ (compare phaseollinisoflavan dimethyl ether [3] 5·77 and 6·09) and the C-4 protons as a broad doublet at 7·06 τ and 7·19 τ (compare 7·06, 7·16). The presence of an *iso*pentenyl side chain is demonstrated by singlets at 8·20 τ and 8·26 τ (methyl groups), a broad triplet at 4·73 τ (methine proton) and a broad doublet at 6·55 τ (methylene protons); the comparable peaks in the spectrum of phaseollidin [4] are found at 8·19, 8·24, 4·72 and 6·64 τ. The NMR spectrum of the new phytoalexin also exhibits signals for one methoxyl group (6·27 τ) and five aromatic protons, indicating a total of four substituent groups. The MS shows the molecular ion at *m/e* 340 and also the retro-Diels Alder fragmentation previously observed in *iso*flavan MS [2, 3, 5], resulting in a peak at *m/e* 218 (97·5% Base Peak). These data require that a single hydroxyl group be assigned to the dihydrobenzopyran moiety of the *iso*flavan, the remaining substituents, now seen to be one methoxyl, one *iso*pentenyl and one hydroxyl group, all being located on the phenyl moiety. On biogenetic grounds the oxysubstituents are assigned to C-7, C-2′ and C-4′ and a negative Gibbs test [6] implies that a hydroxyl group is located at C-4′. The isopentenyl group is situated at C-3′ since signals for H-5′ and H-6′ are observed in the NMR spectrum (doublets at 3·37 τ and 3·09 τ respectively, J 8·7 Hz). The new phytoalexin is therefore 2′-*O*-methylphaseollidinisoflavan (**1**).

On refluxing with dilute H_2SO_4, (**1**) undergoes cyclization of the *iso*pentenyl group with the adjacent hydroxyl group to yield a phenolic *iso*flavan (λ_{max}^{EtOH} 283 nm, M^+ 340) which gives an orange colour with diazotized *p*-nitroaniline.



(**1**)



(**2**)

It is noteworthy that 2′-*O*-methylphaseollidinisoflavan (**1**) could not reliably be distinguished from phaseollidin (**2**) by TLC. Identical R_f values were obtained after development in four solvent systems; only with C_6H_6:EtOAc 3:1 was there any difference in R_f (*c.* 0·1). The UV spectra, however, do show slight but significant differences: (**1**), λ_{max}^{EtOH} 282·5 nm (log ϵ 3·80), 288 sh (3·68); (**2**), 281·5 (3·76),

Table 1. Occurrence of 2'-*O*-methylphaseollidinisoflavan (**1**) and phaseollidin (**2**) in infected cowpea stems

Inoculum	I.I.T.A. Accession line: TVu 32	TVu 37	TVu 76	TVu 57	TVu 317	TVu 400	TVu 2366	TVu 2398
Colletotrichum lindemuthianum isolate I 47 (ex I.I.T.A.)	—	—	—	(1)	(1)	(1)	(1)	
Tobacco necrosis virus	(2)	(1)	(2)*	(1)	(1)	(1)		(1)

* See Ref. 1.

287 (3·80) [3]. In view of the reported occurrence of phaseollidin in cowpea line TVu 76 following inoculation with tobacco necrosis virus [1], seven additional lines were examined qualitatively for the presence of (**1**) and (**2**), with the results shown in Table 1. Lines TVu 32, 37 and 76 are susceptible [7] to *C. lindemuthianum* and therefore required virus inoculation to give an adequate yield of antifungal compounds; the other lines investigated were classified as resistant. (**1**) and (**2**) were identified by TLC (SiO_2, $CHCl_3$:C_2H_5OH 97:3 and C_6H_6:$MeCOOC_2H_5$ 3:1) and by their UV spectra. It is appreciated that mixtures of (**1**) and (**2**) might escape identification by these methods if the minor component is present only in trace quantities.

Compound (**1**) totally inhibited conidiospore germination of Nigerian isolates I 47 and I 57 (ex I.I.T.A.) of *C. lindemuthianum* at 10 and 15 ppm respectively; the values for compound (**2**) were 20 and 25 ppm.

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TYRAMINE FROM *MAGNOLIA* SPECIES

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In Chinese medicine, Shin-i, prepared from dried young buds of *Magnolia* plants, is used as a sedative or an analgesic. In Japan, Shin-i taken internally for treatment of headaches or colds are also young buds of *M. kobus* or *M. salicifolia*. In addition, *M. obovata* is utilized in Japan for abdominal distention or pains, and as a diuretic, and *M. grandiflora* for headache or giddiness.