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5,7,2′,4′-TETRAHYDROXY-8-(3″-HYDROXY-3″-METHYL-BUTYL) ISOFLAVANONE, A METABOLITE OF KIEVITONE PRODUCED BY *FUSARIUM SOLANI* f.sp. *PHASEOLI*

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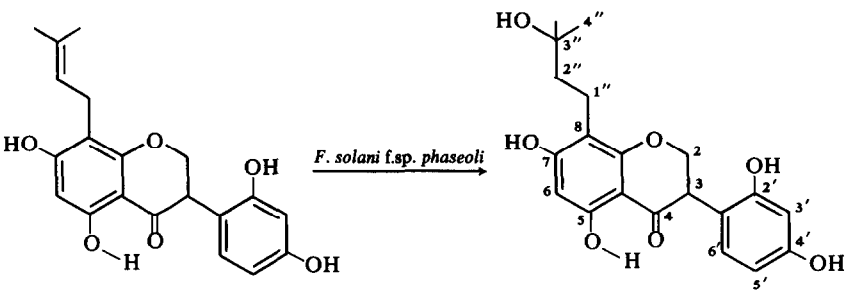
Key Word Index—*Phaseolus vulgaris*; *Fusarium solani* f.sp. *phaseoli*; Leguminosae; fungal metabolism; isoflavonoids; phytoalexins; kievitone; 5,7,2′,4′-tetrahydroxy-8-(3″-hydroxy-3″-methyl-butyl) isoflavanone; kievitone hydrate; detoxification.

INTRODUCTION

Fusarium solani (Mart.) Sacc. f.sp. *phaseoli* (Burk.) Snyd. and Hans., a pathogen of French bean (*Phaseolus vulgaris*) is relatively insensitive to the phytoalexins produced by *P. vulgaris* [1, 2]. The tolerance of *F. solani* f.sp. *phaseoli* to the bean phytoalexin phaseollin, a pterocarpan [3, 4], has been attributed to its ability to metabolize phaseollin to a less fungitoxic product, 1a-hydroxyphaseollone [5, 6]. The metabolism of phytoalexins by plant pathogenic fungi is now well established but, to date, pterocarpans are the only isoflavonoid phytoalexins for which structures of fungal metabolites are known [7]. Kievitone (**1**), an isoflavanone [8, 9] and another of the five phytoalexins produced by *P. vulgaris* [7], is also metabolized by *F. solani* f.sp. *phaseoli* to a less fungitoxic compound [10]. The metabolite is a hydrated product of kievitone and this paper presents evidence that its structure is (**2**).



(1) (2)

RESULTS AND DISCUSSION

The metabolite, 'kievitone hydrate', was isolated in mg amounts from liquid cultures of *F. solani* containing kievitone (21.6 mg of kievitone yielded 14.3 mg of kievitone hydrate). The purified material had lower R_f values than kievitone on Si gel chromatograms [10], but yielded a similar orange-brown reaction product with diazotized *p*-nitroaniline. Its UV[λ_{max}^{EtOH} (log ε) 214 (4.39) and 293 (4.22) nm; shoulder 340 (3.5) nm] and IR [KCl disc: OH band, 3350 cm^{-1}; carbonyl band, 1642 cm^{-1}] spectra were virtually identical to those of kievitone [9]. Low and high resolution MS (obtained using a heated direct insertion probe) revealed a M^+ at *m/e* 374.1373 (15%, $C_{20}H_{22}O_7$), 18 amu higher than kievitone [8, 9], indicating the addition of water to kievitone. Other major peaks in the MS occurred at *m/e* 356 (6%), 300 (64%), 221 (65%), 205 (15%), 192 (14%), 177 (26%) and 165 (100%), all consistent with the fragmentation observed for kievitone [9].

The structure of (**2**) was unequivocally established as 5,7,2′,4′-tetrahydroxy-8-(3″-hydroxy-3″-methyl-butyl) isoflavanone from a comparison of the 100 MHz PMR spectra in DMSO-d_6 and MeOH-d_4 with the reported data for kievitone [9] (Table 1). The olefinic proton H-2″ and the methylene proton H-1″ of kievitone were totally absent in the spectrum of kievitone hydrate and were replaced by two multiplets which showed the typical AA′XX′ structure expected for a X-CH_2-CH_2-Y

fragment. The chemical shifts were typical for methylene in an aliphatic chain (1.5 ppm) and methylene substituted by an aromatic ring (2.5 ppm). In MeOH-d_4 this ethane fragment probably has an exclusively *trans* arrangement of substituents since solvation of the C-7 and C-3″

Table 1. Chemical shifts (ppm) of kievitone and kievitone hydrate

Proton	Kievitone hydrate		Kievitone [9]
	MeOH-d_4	DMSO-d_6	DMSO-d_6/$CDCl_3$
H-6	5.95 (*s*)	5.98 (*s*)	6.04 (*s*)
H-1″	2.8 (*m*)	2.5 (*m*)	3.21 (*d*)
H-2″	1.6 (*m*)	1.5 (*m*)	5.18 (*t*)
H-4″	1.25 (*s*)	1.15 (*s*)	1.64 (*s*)
Me-3″			1.74 (*s*)
H-2a	4.5 (*m*)	4.45 (*m*)	4.49 (*m*)
H-2b			4.64 (*m*)
H-3	4.15 (*dd*)	4.15 (*dd*)	4.16 (*dd*)
H-3′	6.31 (*d*)	6.3 (*d*)	6.45 (*d*)
H-5′	6.25 (*dd*)	6.15 (*dd*)	6.32 (*dd*)
H-6′	6.97 (*d*)	6.78 (*d*)	6.91 (*d*)

hydroxyl groups will destroy any hydrogen bonding between these OH groups; such hydrogen bonding is the only factor which could offset the normal steric repulsions which reduce the population of the *gauche* conformer. A *trans* conformation about the bond between C-1″ and C-2″ implies a molecular shape for kievitone hydrate closely similar to that of its precursor kievitone. The nonequivalent Me groups of kievitone (1) were replaced by a 6-proton singlet in the spectrum of (2), indicating that hydration of the C=C group of kievitone had occurred in a Markovnikov fashion to yield a 3″-OH rather than a 2″-OH side chain. The presumably enzymatically-catalysed hydration was highly specific since no evidence of the system with a 2″-OH side chain was found in the spectrum.

In MeOH-d_4 all OH protons appeared as a single peak at 4.7 ppm due to rapid exchange. Some exchange was also evident in DMSO-d_6 since the phenolic protons for the 7-OH, 2′-OH and 4′-OH groups appeared as a very broad peak at *ca* 9.5 ppm. The aliphatic tertiary OH group was present as a very broad absorption around 3.8 ppm. A sharp singlet at 12.3 ppm (compared with 12.2 ppm in kievitone) confirmed the presence of the strongly intramolecular-hydrogen-bonded OH at C-5.

Preliminary bioassays of kievitone hydrate against *Cladosporium herbarum* (Pers.) Link. [10], *Aphanomyces euteiches* Drechs. and *Rhizoctonia solani* Kühn indicated that this metabolite was considerably less fungitoxic than kievitone. The metabolic conversion of kievitone to kievitone hydrate by *F. solani* f.sp. *phaseoli*, therefore, may well explain the comparative insensitivity of this fungus to the phytoalexin.

EXPERIMENTAL

Kievitone was obtained following procedures outlined previously [2]. Actively growing mycelium of *F. solani* f.sp. *phaseoli* was obtained as described elsewhere [5]. Kievitone dissolved in EtOH was added to fungal cultures at concns of 25–50 μg/ml; the final EtOH conc in the medium was 0.5%. The cultures were incubated at 25° on a reciprocal shaker for 6 hr, after which kievitone hydrate was isolated by slight modification of methods reported elsewhere [5]. The metabolite was purified by sequential TLC on Si gel G developed with C_6H_6–MeOH (9:1) (R_f 0.16) and toluene–ethyl formate–HCO_2H (7:2:1) (R_f 0.07). Further purification was obtained by gel filtration through Sephadex LH-20 employing EtOH as the eluant. Bioassays were carried out as described in refs. [2, 10].

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REFERENCES

1. VanEtten, H. D. and Smith, D. A. (1975) *Physiol. Plant Pathol.* **5**, 225.
2. Smith, D. A. (1976) *Physiol. Plant Pathol.* **9**, 45.
3. Cruickshank, I. A. M. and Perrin, D. R. (1963) *Life Sci.* **2**, 680.
4. Perrin, D. R. (1964) *Tetrahedron Letters* **1**, 29.
5. Van den Heuvel, J. and VanEtten, H. D. (1973) *Physiol. Plant Pathol.* **3**, 327.
6. Van den Heuvel, J., VanEtten, H. D., Serum, J. W., Coffen, D. L. and Williams, T. H. (1974) *Phytochemistry* **13**, 1129.
7. VanEtten, H. D. and Pueppke, S. G. (1976) Isoflavonoid phytoalexins. In *Biochemical Aspects of Plant–Parasite Relationships* (Friend, J. and Threlfall, D. R. eds.). Academic Press, New York (In Press).
8. Burden, R. S., Bailey, J. A. and Dawson, G. W. (1972) *Tetrahedron Letters* 4175.
9. Smith, D. A., VanEtten, H. D., Serum, J. W., Jones, T. M., Bateman, D. F., Williams, T. H. and Coffen, D. L. (1973) *Physiol. Plant Pathol.* **3**, 293.
10. Kuhn, P. J. and Smith, D. A. (1976) *Proc. Am. Phytopath. Soc.* **3**, (In press).

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ANTHOCYANIN COMPOSITION OF *BRASSICA OLERACEA* CV. RED DANISH

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Key Word Index—*Brassica oleracea* cv. Red Danish; Cruciferae; anthocyanins; malonyl-, *p*-coumaryl-, feruloyl-, sinapyl esters of cyanidin-3-sophoroside-5-glucoside.

Abstract—Eight anthocyanins were isolated from illuminated red cabbage seedlings. They were identified as: cyanidin-3-sophoroside-5-glucoside, cyanidin-3-malonyl-sophoroside-5-glucoside, cyanidin-3-*p*-coumaryl-sophoroside-5-glucoside, cyanidin-3-(di-*p*-coumaryl)sophoroside-5-glucoside, cyanidin-3-feruloyl-sophoroside-5-glucoside, cyanidin-3-(diferuloyl)sophoroside-5-glucoside, cyanidin-3-sinapylsophoroside-5-glucoside, and cyanidin-3-(disinapyl)-sophoroside-5-glucoside.