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IDENTIFICATION OF 1*a*-HYDROXY PHASEOLLONE, A PHASEOLLIN METABOLITE PRODUCED BY *FUSARIUM SOLANI*

J. VAN DEN HEUVEL,* H. D. VAN ETTEN and J. W. SERUM

Plant Pathology Department and Department of Chemistry, Cornell University, Ithaca, NY 14850, U.S.A.

and

D. L. COFFEN and T. H. WILLIAMS

Chemical Research Department, Hoffmann-LaRoche Inc., Nutley, NJ 07110, U.S.A.

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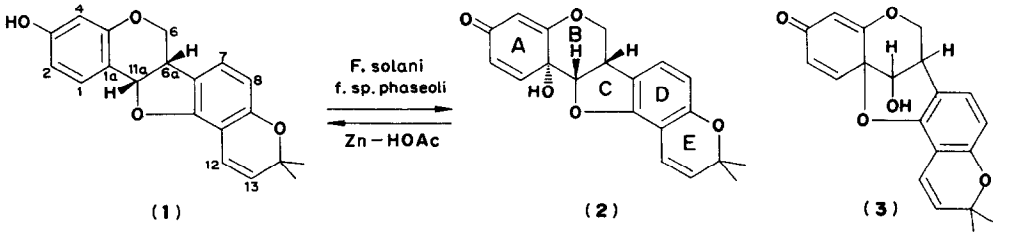
Key Word Index—*Phaseolus vulgaris*; Leguminosae; 1*a*-hydroxyphaseollone; phaseollin; pterocarpan; phytoalexin; antifugal compounds; *Fusiarium solani* f. sp. *phaseoli*; detoxification; phenol metabolism.

Abstract—A structure for the phaseollin metabolite of *Fusarium solani* f. sp. *phaseoli* has been proposed and assigned the name 1*a*-hydroxyphaseollone. The data are consistent with a hydroxylation at position 1*a* with concomitant dienone formation in ring A of phaseollin.

INTRODUCTION

PREVIOUS studies have demonstrated that fungal pathogens of bean, *Phaseolus vulgaris* L., are generally more tolerant of phaseollin (**1**), a phytoalexin from bean, than non-pathogens.[1,2] The bean pathogen *Fusarium solani* (Mart) Sacc. f. sp. *phaseoli* (Burk) Snyd. and Hans. is tolerant of phaseollin, apparently because it metabolizes this phytoalexin to a less fungitoxic compound.[3] This metabolite ("compound B") is an oxidative product of phaseollin and this paper presents evidence that its structure is (**2**).



(1) (2) (3)

RESULTS

"Compound B[4]" is a yellow-brown colored solid that is stable to acid but sensitive to basic conditions. It exhibits UV absorption at [λ_{max}^{EtOH} (log ε)] 228·5 (4·49), 280 (3·98) and 290 (3·95) with a broad shoulder at 208–213 (4·36) and 303–313 (3·75) nm. The optical

* Present address: Phytopathological Laboratory, "Willie Commelin Scholten", Baarn, The Netherlands.

[1] CRUICKSHANK, I. A. M. and PERRIN, D. R. (1971) *Phytopathol. Z.* **70**, 209.

[2] VAN ETTEN, H. D. (1973) *Phytopathology* **63**, 1477.

[3] HEUVEL, J. VAN DEN and VAN ETTEN, H. D. (1973) *Physiol. Plant. Path.* **3**, 327.

rotation of this compound is $[\alpha]_D^{24}$ −187° (MeOH) while that of phaseollin[4] is $[\alpha]_D^{24}$ −121° (MeOH). Unlike phaseollin, "compound B" reacts with 2,4-dinitriphenylhydrazine to give a yellow colored product and exhibits a prominent IR absorption ($CHCl_3$) at 1665 cm^{-1}, both results indicative of a carbonyl group. Although broad IR absorption at 3290 cm^{-1} suggests the presence of a OH group, "compound B" reacts only weakly with diazotized *p*-nitroaniline and is not readily acetylated with acetic anhydride/pyridine nor methylated with diazomethane; no colored products are obtained with $FeCl_3$. These findings suggest the lack of a phenolic hydroxyl group.

High and low resolution MS (obtained with an AEI M.S. 902 instrument using a heated direct insertion probe) indicate a parent ion at *m/e* 338, 16 m.u. higher than phaseollin, and an elemental composition of $C_{20}H_{18}O_5$. The base peak in the mass spectrum is at *m/e* 323 ($C_{19}H_{15}O_5$, 100%) and is consistent with the presence of a 2,2-dimethylchromene ring as in the spectrum of phaseollin.[5] The peaks at *m/e* 213 ($C_{14}H_{13}O_2$, 11%) and *m/e* 185 ($C_{12}H_9O_2$, 9%) may be formed by cleavage of the B ring in the latter case accompanied by an additional methyl loss presumably from the dimethyl chromene

TABLE 1. ASSIGNMENTS OF CHEMICAL SHIFTS AND COUPLING CONSTANTS OF 1*a*-HYDROXYPHASEOLLONE (COMPOUND B) AND PHASEOLLIN

Protons	Compound		
	"Compound B"	Phaseollin[4]	Phaseollin[5]
H-1	6·69 *d*, *J* 10	7·40 *d*, *J* 8·5	7·37
H-2	6·09 broadened *d*, *J* 10	6·53 *dd*, *J* 2, 8·5	6·54
H-4	5·48 *d*, *J* 2	6·40 *d*, *J* 2	6·41
CH_2-6	5·08 *dd*, *J* 4, 10 4·33 broadened *d*, *J* 10	4·23 3·61	4·20 3·64
H-6*a*	3·91 broadened *dd*, *J* 4, 10	3·49	3·50
H-7	6·88 *d*, *J* 8·5	6·94 *d*, *J* 8	6·92
H-8	6·32 *d*, *J* 8·5	6·34 *d*, *J* 8	6·32
H-11*a*	5·14 sharp *d*, *J* 10	5·47 *d*, *J* 7	5·55
H-12	6·22 *d*, *J* 10	6·50 *d*, *J* 10	6·48
H-13	5·48 *d*, *J* 10	5·56 *d*, *J* 10	5·54
$(Me)_2$	1·37 (1·39 and 1·42 in $CDCl_3$–DMSO-d_6)	1·39 1·42	1·49 1·52

[4] "Compound B" was isolated as described in Ref. 3. The phaseollin used in this study for comparative purposes was obtained by the procedure of VANETTEN, H. D. and BATEMAN, D. F. (1970) *Phytopathology* **60**, 385.

[5] PERRIN, D. R., WHITTLE, C. P. and BATTERHAM, T. J. (1972) *Tetrahedron Letters* 1673.

ring. These cleavages are analogous to pterocarpin,[6] and suggest that rings C, D and E remain unchanged from the phaseollin structure. A moderately intense peak is observed at *m/e* 161 ($C_{10}H_9O_2$, 25%). This fragment may be rationalized by cleavage of the C ring with charge retention on the chromene moiety. The absence of an ion at *m/e* 147, which is thought to be formed as a hydroxybenzopyrylium ion from rings A and B in phaseollin[5] also lends support to the structure being altered in ring A.

Location of the carbonyl and hydroxy functional groups was accomplished by comparison of the NMR spectra of "compound B" and phaseollin (recorded in $CDCl_3$ with TMS as internal standard) (Table 1). The lack of low-field phenolic OH signals confirms the absence of this functional group. Analysis of the spectrum is straightforward and leads, in conjunction with the other data, directly to the hypothesis that "compound B" has structure **2**, the product resulting from hydroxylation at position 1*a* with concomitant dienone formation in ring A. This hypothesis was corroborated by the observation that stirring with zinc dust in acetic acid effects clean reduction back to phaseollin (**1**).[7] We propose that structure **2** be assigned the name 1*a*-hydroxyphaseollone.

An alternate structure could conceivably be **3**. However the value of $J_{6a,11a}$ is 10 Hz. This is found in the fused ring system of **2** but it is unlikely to be this high in the bridged system **3** where 2–5 Hz is the normal range of *J*.

It is not possible to make an unequivocal assignment of relative stereochemistry in 1*a*-hydroxyphaseollone on the basis of the spectral data in hand. However we favor that shown in structure **2** for the following reason. The protons at positions 6, 6*a*, and 11*a* form a four-spin system in which, unlike phaseollin, no one proton is split by two large (10 Hz) *J* values. Therefore the methylene protons are both *gauche* to the methine proton at 6*a* requiring that this proton be equatorial to ring B rather than axial, as in phaseollin and related pterocarpans.[8] *Endo* hydroxylation of phaseollin leading to structure **2** would induce the proposed flip of ring B whereas *exo* hydroxylation would leave the conformation of this ring unchanged.

This type of oxidative detoxification of phenolic compounds would appear to be an unusual means for the metabolism of phenols. Normally, monohydroxyphenols are oxidized by *ortho* hydroxylation followed by an oxidation of the *o*-dihydroxyphenol.[9] This mechanism of detoxification of phaseollin by *F. solani* f. sp. *phaseoli* may suggest a more specialized adaptation of this organism to phaseollin.

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[8] PACHLER, K. G. R. and UNDERWOOD, W. G. E. (1967) *Tetrahedron* **23**, 1817.
[9] TOWERS, G. H. N. (1964) in *Biochemistry of Phenolic Compounds* (HARBORNE, J. B., ed.), p. 249, Academic Press, London.