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SHORT COMMUNICATION

HYDROXYPHASEOLLIN, AN INDUCED ANTIFUNGAL COMPOUND FROM SOYBEANS

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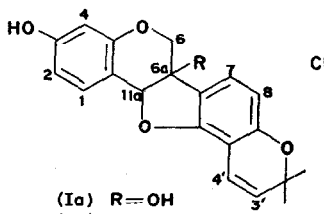
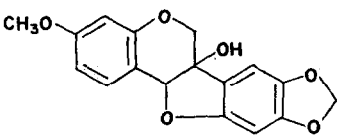
Abstract—The antifungal compound produced by soybeans in response to infection by the fungus *Phytophthora megasperma* is assigned structure Ia on the basis of spectral and chemical evidence.

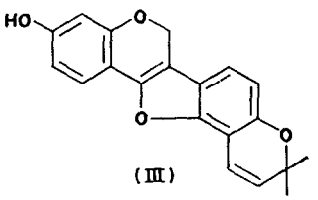
PREVIOUS work by Klarman and Sanford[1] has established that a single antifungal compound is produced in the host-parasite interaction between soybeans and certain *Phytophthora* species.[2] We have isolated this antifungal compound from soybean hypocotyls[3] that were inoculated with *Phytophthora* and evidence presented here shows that its structure is represented by (Ia). Phaseollin (Ib) a related antifungal compound has been isolated from french bean tissue.[4]



(Ia) R=OH
(Ib) R=H



(II)



(III)

Hydroxyphaseollin (Ia) was isolated by preparative TLC[3] as a viscous oil, $[\alpha]_D^{20}$ −207° (ETOAC). It shows UV absorption at $[\lambda_{max}^{EtOH}$ (log ϵ)] 206 (4·57), 227 (4·43), 285 (3·92), 290 sh (3·88), 305 sh (3·39) and 318 sh (3·23) nm. As is typical of phenols, a shift of UV absorption takes place in alcoholic NaOH to give λ_{max} 293 (4·12) and 318 (3·49) nm. The UV data for hydroxyphaseollin are very much the same as for phaseollin[5] and similar to other related compounds. The high negative rotation of Ia is also common among related pterocarpans.[5] The presence of hydroxyl absorption and the lack of any carbonyl absorption was shown by the IR spectrum.

The mass spectrum of Ia gave a parent ion at *m/e* 338, which agrees with the empirical formula $C_{20}H_{18}O_5$. Other intense peaks were found at *m/e* 323 (*p*-CH_3, base peak), 320 (*p*-H_2O), 305 (*p*-CH_3–H_2O), 293 (*p*-CH_3–CO), 277 (*p*-CH_3H_2O–CO). Also observed were doubly charged ions at *m/e* 161·5 (323^{2-}), 160 (320^{2+}), 152·5 (305^{2+}) and 138·5 (277^{2+})

[1] W. L. KLARMAN and J. D. SANFORD, *Life Sciences* **7**, 1095 (1968).
[2] W. L. KLARMAN and J. W. GERDEMAN, *Phytopathology* **53**, 863, 1317 (1963).
[3] N. T. KEEN, J. J. SIMS, D. C. ERWIN, E. RICE and J. E. PARTRIDGE, *Phytochem.* (in press).
[4] D. R. PERRIN, *Tetrahedron Letters* 29, (1964).
[5] I. A. M. CRUICKSHANK and D. R. PERRIN, *Life Sciences* **3**, 680 (1963).

which are characteristic of condensed aromatic systems and have been observed in the mass spectrum of Ib.[6] In general the mass spectrum of Ia resembles that of Ib with the addition of 1 oxygen atom as a hydroxyl group. The new fragments are due to the ready loss of H_2O from the parent ion.

The NMR spectrum of Ia in $CDCl_3$ showed a 6H singlet at δ 1·40 (gem dimethyl at C_{21}), a broad 4H multiplet centered at δ 4·00 (2OH + $2H_6$), a sharp, 1H singlet at δ 5·24 (H_{11a}), a 1H doublet at δ 5·55 (H3', $J = 10$ Hz), a 4H multiplet δ 6·14–6·76 (H_2, H_4, H_8, H4'), and a pair of overlapping, doublets centered at δ 7·17 (H_7, H_1). These data are consistent with the placement of the non-phenolic hydroxyl group, inferred from the mass spectrum, at C-6a. The hydrogen at C-11a in 1a is clearly a singlet, differing from the doublet found for the same hydrogen in Ib.[4] With pisatin (II) we have observed[7,8] a similar singlet at δ 5·23 for the IIa proton.

The chemical behavior of hydroxyphaseollin confirmed structure Ia. Acetylation with pyridine/acetic anhydride produced a monoacetate (parent ion *m/e* 380); as would be expected, the tertiary alcohol was unreactive. Treatment with acid gave the dehydration product III analogous to the similar compound formed from pisatin.[9] Compound III was characterized by its mass spectrum which showed a parent ion at *m/e* 320 plus strong peaks for loss of methyl and CO. The compound was optically inactive as would be expected.

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[6] Dr. HANS VAN ETTEN, Cornell University, kindly loaned us a sample of phaseollin and a copy of its mass spectrum.
[7] We are grateful to Professor LEE HADWIGER and Dr. M. SCHWOCHAU, Washington State University, for providing a sample of pisatin.
[8] D. D. PERRIN and D. R. PERRIN, *J. Am. Chem. Soc.* **84**, 1922 (1962).
[9] D. R. PERRIN and W. BOTTOMLEY, *J. Am. Chem. Soc.* **84**, 1919 (1962).

Key Word Index—Glycine max; Leguminosae; soybean; antifungal compound; phytoallexin; hydroxyphaseolin.