Phytochemistry, Vol. 28, No. 12, pp. 3317–3319, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
Pergamon Press plc

ANTIFUNGAL ACTIVITY OF ISOFLAVONOIDS AGAINST STORAGE FUNGI OF THE GENUS *ASPERGILLUS*

MARTIN WEIDENBÖRNER, HOLGER HINDORF, HEM CHANDRA JHA,* PRODROMOS TSOTSONOS* and HEINZ EGGE*

Institut für Pflanzenkrankheiten der Universität Bonn Nussallee 9, D-5300 Bonn 1, F.R.G.; *Institut für Physiologische Chemie der Universität Bonn Nussallee 11, D-5300 Bonn 1, F.R.G.

(*Received in revised form* 5 *May* 1989)

Key Word Index—*Aspergillus*; storage fungi; isoflavonoids; antifungal activity.

Abstract—The fungicidal activity of two isoflavones, one isoflavanone and seven isoflavans was tested in malt extract broth against five storage fungi of the genus *Aspergillus*. While the isoflavones and isoflavanone show only low activity, the two isoflavans 7,8-dihydroxy-4′-methoxyisoflavan and 6,7-dihydroxy-3′-methylisoflavan were highly inhibitory to *Aspergillus*. Structure–activity relationships are discussed.

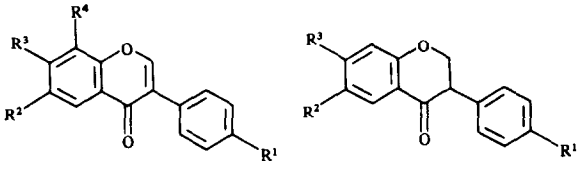
INTRODUCTION

Isoflavonoids are products of secondary plant metabolism of legumes [1] whose antimicrobial effects have been extensively studied [2–7]. The utilization of these natural compounds as substitutes for conventional fungicides in the prevention of plant diseases has been considered [8, 9]. However, to find suitable fungicides in this group of isoflavonoids *in vitro* screenings and the establishment of structure–activity relationships are essential. Such a study may be of immense use in developing some of the isoflavonoids as suitable antifungal substances against storage fungi of the genus *Aspergillus*. The following investigation presents further data in continuation of our previous work in this field [6, 10, 11].

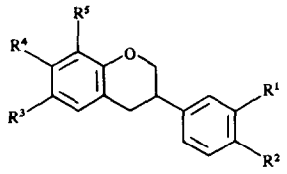
RESULTS AND DISCUSSION

The three isoflavonoids **1–3** showed weak inhibitory activities. Contrary to our previous experience, the isoflavan **3** stimulated the growth of *A. repens* and *A. petrakii*. When compared with **1**, the isoflavone **4** is a stronger inhibitor of fungal growth. The isoflavan **5** caused 70.3 and 86.9% growth inhibition of *A. amstelodami* and *A. chevalieri*. respectively. The growth inhibition up to 89.5% of *A. repens* at concentration *c* was statistically not significant. The isoflavan **6** exhibited growth stimulation in the case of the fungi of the *A. glaucus* group (*A. repens*, *A. amstelodami*, *A. chevalieri*) at concentration *a*, but at higher concentrations it was inhibitory. The isoflavan **7** inhibited the growth of *A. petrakii* up to 51.2 and 58.8% at the concentrations *b* and *c*, respectively. The other fungi *A. amstelodami* and *A. chevalieri* were inhibited weakly. The isoflavan **8** was found to be a poor inhibitor of fungal growth. Of all the tested compounds the isoflavan **9** showed the highest activity with all the fungi studied. At concentration *c* it inhibited the growth of fungi up to 98% (excepting *A. petrakii* which was inhibited only up to 73.2%). The isoflavan **10** showed lower inhibitory activity.

Although our previous investigations have demonstrated that isoflavans, in general, possess higher activity



1 $R^1 = R^2 = R^3 = OH$, $R^4 = H$ **2** $R^1 = R^2 = R^3 = OH$
4 $R^1 = OMe$, $R^2 = H$, $R^3 = R^4 = OH$



3 $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = OH$
5 $R^1 = R^3 = H$, $R^2 = OMe$, $R^4 = R^5 = OH$
6 $R^1 = R^3 = R^5 = H$, $R^2 = R^4 = OH$
7 $R^1 = R^2 = R^5 = H$, $R^3 = OH$, $R^4 = OMe$
8 $R^1 = R^2 = OMe$, $R^3 = R^4 = OH$, $R^5 = H$
9 $R^1 = Me$, $R^2 = R^5 = H$, $R^3 = R^4 = OH$

10 $R^1 = R^5 = H$, $R^2 = OMe$, $R^3, R^4 = OCH_2O$

than the corresponding isoflavones and isoflavanones [10], all three isoflavonoids **1–3** show a uniform level of low activity. This and other results [7, 12] are contrary to the conclusion that the antifungal activity depends on the skewed, aplanar molecular shape of the isoflavans and pterocarpans [13]. Meanwhile our own results [11, and unpublished results] and those of Van Etten [7] have been able to show that neither the molecular shape nor the degree of reduction is exclusively responsible for inhibitory activity. The low activity of 6,7,4′-trihydroxyisoflavan (**3**) [10] as well as of 6,2′,4′ and 2′,4′,6′-trihydroxy-2-phenylbenzofuran [14] indicates that several hydroxyl groups in the molecule are not conducive to antifungal activity. Ward *et al.* [15] obtained similar results with trihydroxydihydrophenathrene. It seems that

the hydroxyl groups contribute to the higher polarity of the molecule which may minimize the fungal membrane permeability of the substances. Smith [16] and Adesanya *et al.* [17] found kievitone to be very effective against different fungal species. This activity was reduced when the more polar kievitone hydrate was examined. Also the oxidation of the lipophilic side chain of kievitone to the hydrophilic primary alcohol resulted in decreased activity. However, 7,2′,4′-trihydroxyisoflavan (demethylvestitol) is fungitoxic to *Cladosporium herbarum* and *Aspergillus* niger [17]. It appears that, in addition to the number of polar groups, the position of the substituents in the molecule exerts an influence on the activity. Comparison of the isoflavonoids **4** and **5** with **1**–**3** reveals that in spite of the common *o*-dihydroxylic groups in both series the presence of a methoxy group in **4** and **5** plays a decisive role in conferring activity. The methoxy group may also be important for the stability of the substances against fungal degradation [18]. While the isoflavan **5** is fungitoxic to *A. glaucus* group with 70–87% inhibition, the isoflavone **4** reduces the growth of *A. repens* at concentration *b* only up to 41%. This corroborates our results that isoflavans with two hydroxyl and one methoxy group are highly active irrespective of the particular substitution pattern [6]. The isoflavan **5** only inhibits *A. flavus* slightly, while there is no effect on mycelial growth of *A. petrakii*. By contrast mycelial growth of *A. glaucus* fungi is strongly inhibited. These results show that fungi vary in their susceptibility to growth inhibition by isoflavonoids [19]. The results obtained with the isoflavan **7** indicate that one hydroxyl and one methoxy group in the molecule are insufficient for high activity [10, 20]. On the other hand, the presence of two hydroxyl groups as in case of the 7,4′-dihydroxyisoflavan **6** produces effective inhibition of the test fungi. O'Neill and Mansfield [20] observed similar effects with 7,2′-dihydroxyisoflavan. However, the antifungal effect of 7,4′-dihydroxyisoflavan against *Aspergillus melleus*, *Penicillium digitatum* and *Fusarium culmorum* is rather low [10]. This shows that two hydroxy groups may guarantee some antifungal activity, but only against certain fungi. In the present investigation, the isoflavan **10** had little effect on mycelial growth of the five species of *Aspergillus*. Considering that the fungi may metabolize isoflavonoids [11, 21–23], a transformation of **10** (demethylenation of the methylenedioxy group) might produce the highly active 6,7-dihydroxy-4′-methoxyisoflavan [6]. However, the genus *Aspergillus* does not appear to be capable of such metabolism and no metabolite of this type could be detected in the extract of the culture medium. The fungicidal effect of the isoflavan **8** is also low, probably due to the presence of methoxy groups at 3′- and 4′-positions [20]. The most effective compound of all is the isoflavan **9**. At concentration *c* mycelial growth of all fungi is inhibited up to 70% or more. Whether the methyl group in the B-ring and the two hydroxyl groups in the ring-A of an isoflavan together with the skewed, aplanar molecular shape are responsible for the high activity needs further investigation.

Table 1. Effects of isoflavonoids on mycelial growth of five *Aspergillus* species

	Aspergillus repens			*Aspergillus amstelodami*			*Aspergillus chevalieri*			*Aspergillus flavus*			*Aspergillus petrakii*		
	0.5*	2	8	0.5	2	8	0.5	2	8	0.5	2	8	0.5	2	8
1				−6.0	−6.6					−13.4	−12.0	−9.5			−17.8
2				−14.0	−15.3	−18.8	−13.1		−11.3				−18.7	−24.2	−24.0
3	+12.6					−14.2							+8.6		+14.5
4	−19.9	−41.2	−26.5		−25.2	−25.3									
5						−70.3			−86.9	−14.7	−17.3	−36.8			
6	+12.5		−64.0	+11.5		−54.2	+22.4		−67.2			−10.4			−39.8
7						−14.8	−20.7	−17.8	−33.7				−46.2	−51.2	−58.8
8				+4.5	−4.2							−23.0			
9	+11.7	−68.7	−97.5			−98.3		−33.1	−96.1		+9.9	−94.5			−73.2
10		−18.3							−9.4						

% differences in wt as compared with the control; (+) indicates significant growth stimulation, (−) indicates significant growth inhibition.
*Concentrations are 0.5, 2 and 8×10^{-4} M/l.

EXPERIMENTAL

The fungi used in this investigation were all soil-borne species of the genus *Aspergillus*: *A. repens* de Bary, *A. amstelodami* (Thom & Church) and *A. chevalieri* (Mangin) Thom & Church of the *Aspergillus glaucus* Link group; *A. flavus* Link of the *Asper-*

gillus flavus Link group and *A. petrakii* Vörös of the *Aspergillus ochraceus* Wilhelm group. They were isolated from seeds of soybean, pigeon pea, kidney bean, peanut and cotton [24].

The effect of isoflavonoids on mycelial growth was investigated in liquid culture. The solution contained 30 g malt extract and 3 g peptone 1 dist. H_2O. Me_2CO was used as solvent for the isoflavonoids. The solvent concn in the solution was maintained at 1.1% level. Medium (20 ml) including the particular isoflavonoid in the required concn was transferred to 100 ml flasks and inoculated with five small pieces of mycelium (5 mm in diameter). The flasks were incubated at 23–25° on a reciprocal shaker for 7 days.

Concentrations of isoflavonoids. From phytoalexin experiments [25] it is known that concentrations in the range 10^{-5}–10^{-3} mol/l exhibit fungal inhibition. Therefore the concentrations of 0.5, 2.0 and 8.0×10^{-4} mol/l denoted as *a*, *b* and *c* respectively were used in these tests.

Evaluations of results. Round filter papers (Schleicher & Schüll No. 595) were weighed after drying for 24 hr at 105°. After filtration of the culture the residue was dried and weighed again. The difference in weights gave the dry wt of the fungi. The mean value of 8 repetitions for each concentration and fungus was used for calculation. The data were evaluated by analysis of variance. Probability of single differences was calculated at the 5% level.

Isoflavonoids. 10 isoflavonoids (Table 1) were tested at 3 concns for their effect on mycelial growth of 5 moulds of the genus *Aspergillus*. These isoflavonoids were obtained by synthesis in the Physiological Chemistry Institute of the University of Bonn, and their purity and authenticity were verified by physical methods (NMR and MS).

REFERENCES

1. Ingham, J. L. (1984) in *Phytoalexins* (Bailey, J. A. and Mansfield, J. W., eds), p. 21. Blackie, Glasgow.
2. Bailey, J. A. and Skipp, R. A. (1978) *Ann. Appl. Biol.* **89**, 354.
3. Fraile, A., Garcia-Arenal, F., Garcia-Serano, J. J. and Sagasta, E. M. (1982) *Phytopathol. Z.* **105**, 161.
4. Gnanmanickam, S. S. and Smith, D. A. (1980) *Phytopathology* **70**, 894.
5. Kaplan, N. T. and Thomason, I. J. (1980) *Physiol. Pl. Pathol.* **16**, 309.
6. Weidenbörner, M., Jha, H. C., Hindorf, H., Weltzien, H. C. and Zilliken, F. (1987) *Med. Fac. Landbouww. Rijksuniv. Gent* **52**, 933.
7. Van Etten, H. D. (1976) *Phytochemistry* **15**, 655.
8. Rathmell, W. G. and Smith, D. A. (1980) *Pestic. Sci.* **11**, 568.
9. Mansfield, J. W. and Bailey, J. A. (1984) in *Phytoalexins* (Bailey, J. A. and Mansfield, J. W., eds), p. 319. Blackie, Glasgow.
10. Krämer, R. P., Hindorf, H., Jha, H. C., Kallage, J. and Zilliken, F. (1984) *Phytochemistry* **23**, 2203.
11. Weidenbörner, M., Jha, H. C. and Hindorf, H. (1988) *Med. Fac. Landbouww. Rijksuniv. Gent* **53**, 1417.
12. Van Etten, H. D. and Pueppke, S. G. (1976) in *Biochemical Aspects of Plant–Parasite Relationships* (Friend, J. and Threlfall, D. R., eds), *Ann. Proc. Phytochem. Soc.* **13**, 239.
13. Perrin, D. R. and Cruickshank, I. A. M. (1969) *Phytochemistry* **8**, 971.
14. Carter, G. A., Chamberlain, K. and Wain, R. L. (1978) *Ann. Appl. Biol.* **88**, 57.
15. Ward, E. W. B., Unwin, C. H. and Stoessl, A. (1975) *Can. J. Botany* **53**, 964.
16. Smith, D. A. (1978) *Phytochemistry* **68**. 81.
17. Adesanya, S. A., O'Neill, M. J. and Roberts, M. F. (1986) *Physiol. Pl. Pathol.* **29**, 95.
18. Sharma, R. D. (1979) *Lipids* **14**, 535.
19. Van Etten, H. D., Matthews, D. E. and Smith, D. A. (1984) in *Phytoalexins* (Bailey, J. A. and Mansfield, J. W., eds), p. 200. Blackie, Glasgow.
20. O'Neill, T. M. and Mansfield, J. W. (1982) *Trans. Br. Mycol. Soc.* **79**, 229.
21. Kraft, B. and Barz, W. (1985) *Appl. Envir. Microbiol.* **50**, 45.
22. Kraft, B., Schwenen, L., Stöckl, D. and Barz, W. (1987) *Arch. Microbiol.* **147**, 201.
23. Kallage, J. (1984) Dissertation, University of Bonn.
24. Weidenbörner, M. and Hindorf, H. (1989) *Seed Sci. Technol.* **15**, (in press).
25. Van Etten, H. D. and Bateman, D. F. (1971) *Phytopathology* **61**, 1363.