Acta Bot. Neerl. 29 (2/3), May 1980, p. 193–197.

BRIEF COMMUNICATIONS

BIOLOGICAL IMPLICATIONS OF THE STEREOCHEMISTRY OF ISOFLAVONOID PHYTOALEXINS

A. FUCHS and C. A. X. G. F. SICHERER*

Laboratoriumvoor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, 6709 PD Wageningen**

To properly evaluate the role of phytoalexins in plant disease resistance it is useful to know whether or not the pathogens involved are capable of metabolizing and detoxifying these compounds. In studying this question, rather than the mechanism of degradation *per sé* the specificity of fungi in degrading phytoalexins seems crucial.

For several years we have been studying the *in vitro* breakdown of pterocarpanoid phytoalexins by various fungal pathogens (for recent literature, see FUCHS & HIJWEGEN 1979; FUCHS et al. 1980a, b). The pterocarpan skeleton with its numbering system is given in *fig. 1a;* the most common sites of substitution in the different pterocarpans are the 2, 3, and 4-positions in the A-ring, the 8, 9, and 10-positions in the D-ring and the 6a-position.

Due to the two asymmetric carbon atoms (centres of chirality) and the *cis*-fusion of the B and C-ring, as evident from NMR data (PACHLER & UNDERWOOD

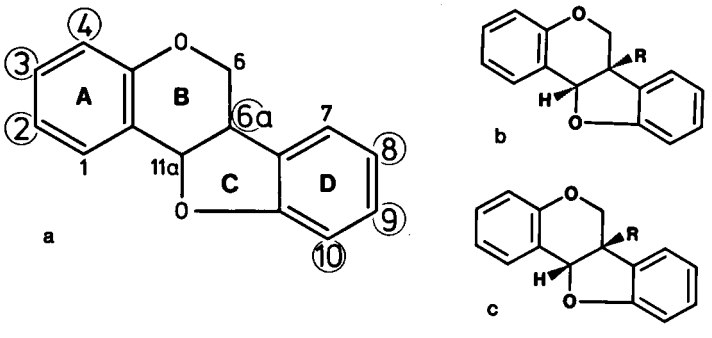

Fig. 1. a. Pterocarpan skeleton with preferred sites of substitution (in circles); b. (–)-pterocarpan with 6aα, 11aα-configuration (R = H: 6aR, 11aR; R = OH: 6aS, 11aS); c. (+)-pterocarpan with 6aβ, 11aβ-configuration (R = H: 6aS, 11aS; R = OH: 6aR, 11aR).

*Present address: Laboratorium voor Organische Chemie, Technische Hogeschool, Delft, The Netherlands.

**Communicated at the Meeting of the section for Plant Pathology (Royal Botanical Society of the Netherlands) on November 22, 1979.

1967), all pterocarpans occur in two enantiomeric forms (*figs. 1b, c*). In general, to designate absolute configurations of pterocarpans the R, S nomenclature is used. With this nomenclature it depends on the nature of the substituent at the C-6a position whether a certain absolute configuration is indicated as 6aR, 11aR or 6aS, 11aS (SICHERER & SICHERER-ROETMAN 1980). With the α, β nomenclature, on the other hand, such a confusion is avoided, though the α, β nomenclature is not *a priori* unambigious. According to this designation, irrespective of the nature of the substituent at C-6a a pterocarpan as depicted in *fig. 1b* has the 6aα, 11aα-configuration, with a negative value of $[\alpha]_D^{\cdot}$, whereas the configuration as given in *fig. 1c* represents the 6aβ, 11aβ-configuration, with a positive value of $[\alpha]_D$ (see *table 1*, which summarizes substituents and absolute configuration at C-6a and C-11a for some common pterocarpans). Generally, of each pterocarpan only one enantiomer is found in nature; however, in some instances two enantiomers co-occur in one plant species (for instance, (+)-inermin (= maackiain) and (±)-inermin (PERRIN & CRUICKSHANK 1969; BRAZ FILHO et al. 1973).

Literature data show that in some fungi differences in substitution pattern do not or hardly interfere with the fungus' ability to carry out a given type of degradation. For instance, the alfalfa pathogen *Stemphylium botryosum* can convert its host phytoalexin medicarpin (STEINER & MILLAR 1974) as well as (–)-inermin (HIGGINS 1975) and phaseollin (HIGGINS et al. 1974) To their corresponding isoflavans by reductive opening of the dihydrofuran ring C (*cf. fig. 2*). Apparently, the substitution pattern at C-8 and 10 in the D-ring (*cf. table 1*) does not determine the substrate specificity of the enzyme(s) involved in this type of degradation.

However, (+)- 6a-hydroxy-inermin, which has a substitution pattern in the A and D-ring similar to that of (–)-inermin, but differs from the latter pterocarpan in its absolute configuration and in possessing a hydroxyl group at C-6a, is not

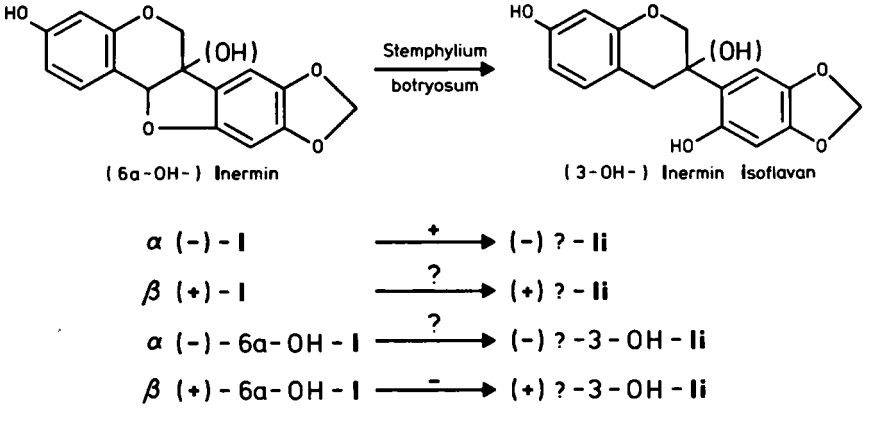

Fig. 2. Conversion of the two enantiomers of inermin and 6a-hydroxy-inermin by *Stemphylium botryosum* (because of the different numbering system of pterocarpans and isoflavans the 6a-position of pterocarpans is comparable with the 3-position of isoflavans).

Table 1. Substituents and absolute configuration at C-6a and C-11a of some common pterocarpans. i.p. = isopentenyl

Pterocarpan	C-2	C-3	C-4	C-6a	abs. conf.	C-8	C-9	C-10
(–) medicarpin	H	OH	H	H	α	H	OCH_3	H
(–) pterocarpin	H	OCH_3	H	H	α	O - CH_2 - O		H
(±) inermin (= maackiain)	H	OH	H	H	α or β	O - CH_2 - O		H
(±) 6a-hydroxy-inermin	H	OH	H	OH	α or β	O - CH_2 - O		H
(+) pisatin	H	OCH_3	H	OH	β	O - CH_2 - O		H
(+) variabilin	H	OCH_3	H	OH	β	H	OCH_3	H
(+) tuberosin	H	OH	H	OH	β	chromene (-O)		H
(–) glyceollin I	H	(O-) chromene		OH	α	H	OH	H
(–) glyceollin II	chromene (-O)		H	OH	α	H	OH	H
(–) phaseollin	H	OH	H	H	α	H	(O-)chromene	
(–) phaseollidin	H	OH	H	H	α	H	OH	i.p.

converted to its corresponding 3-hydroxy-inerminisoflavan (*cf. fig. 2*) by this fungus. On the other hand, depending on the fungus involved a certain pterocarpan might be converted along very different pathways, as exemplified by phaseollin, for which at least five types of conversion are known at this moment (*cf.* VAN ETTEN & PUEPPKE 1976; BAILEY et al. 1977). However, the available knowledge, although limited, on the various pathways of fungal degradation of pterocarpans (for synopsis, see HIJWEGEN & FUCHS, to be published) does suggest that substitution at C-6a as well as absolute configuration are among the key determinants for degradation of pterocarpans by a given fungal species.

Another aspect to be taken into account in the evaluation of the role of phytoalexins is the sensitivity of fungi to these compounds. It should be realized that low or non-sensitivity can be either an intrinsic feature of the fungal species involved or a consequence of rapid breakdown of the phytoalexin concerned. However, the ability to degrade a phytoalexin does not in itself imply non-sensitivity: whereas *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *pisi* are equally sensitive to pisatin in a thin-layer chromatographic bioassay (HOMANS & FUCHS 1970) as well as in a mycelial growth test (FUCHS & DE VRIES, to be published), under normal *in vivo* conditions the pea pathogen apparently circumvents the toxic action of the phytoalexin through rapid degradation (*cf.* FUCHS et al. 1980b). Unlike substrate specificity of fungi with respect to phytoalexin breakdown, sensitivity to phytoalexins does not seem to depend on the absolute configuration at C-6a and C-11a. Although PERRIN & CRUICKSHANK (1969) and VAN ETTEN (1976) disagree with each other about the occurrence of a structure-activity relationship with regard to antifungal activity of pterocarpans, a given absolute configuration evidently is no prerequisite for fungitoxicity: (+)-, (±)- and (–)-inermin inhibited the growth of *Monilinia fructicola* to the same extent.

From the considerations given above it is evident that in such studies as currently performed by us on pterocarpanoid phytoalexin degradation an exact knowledge about absolute configurations is indispensable. Substitution patterns

as well as conformational data on the pterocarpan skeleton may well be derived from NMR-analysis. However, up till now absolute configurations of pterocarpans have usually been established with reference to the absolute configuration of trifolirhizin (= (–)-inermin-β-D-glucoside) as determined by ITO et al. (1965) via degradation to (–)-paraconic acid. Because of the possibly significant role of the absolute configuration at C-6a and C-11a, it seems desirable to use less indirect methods, such as anomalous X-ray scattering, to establish the absolute stereochemistry of pterocarpans. Use of direct methods is also more appropriate in the elucidation of biogenetic relationships among pterocarpans (SICHERER & SICHERER-ROETMAN, 1980) as well as in establishing the stereochemistry of isoflavan analogues of pterocarpans (*cf.* KUROSAWA et al., 1968, 1978). In this context, it is interesting to note that the absolute configuration of (–)-phaseollin as reported by VAN DEN HEUVEL et al. (1974) and DEMARTINIS et al. (1977, 1978) is contrary to current views, though not based on experimental evidence (COFFEN, pers. comm.; DEMARTINIS et al., pers. comm.).

As soon as the absolute configuration of one pterocarpan has unequivocally been established, that of others may be determined by comparison, by means of circular dichroism (CD) or optical rotatory dispersion (ORD), rather than by simple estimation of one optical rotation $[\alpha]_D$ value. For instance, using CD we confirmed that the absolute configuration of pisatin from both *Pisum sativum* and *Lathyrus odoratus* is opposite to that of phaseollin (from bean), glycecollin (from soy bean) and medicarpin (from Jack bean).

REFERENCES

BAILEY, J. A., R. S. BURDEN, A. MYNETT & C. BROWN (1977): Metabolism of phaseollin by Septoria nodorum and other non-pathogens of Phaseolus vulgaris. *Phytochemistry* **16**: 1541–1544.

BRAZ FILHO, R., O. R. GOTTLIEB, S. L. VIEIRA PINHO, F. J. QUEIROZ MONTE & A. I. DA ROCHA (1973): Flavonoids from Amazonian Leguminosae. *Phytochemistry* **12**: 1184–1186.

DEMARTINIS, C., M. F. MACKAY, D. R. PERRIN & B. J. POPPLETON (1977): The molecular structure of phaseollin. *Tetrahedron Lett.* 2981–2982.

—, — & B. J. POPPLETON (1978): The crystal and molecular structure of (–)-phaseollin. *Tetrahedron* **34**: 1849–1852.

FUCHS, A. & T. HIJWEGEN (1979): Specificity in degradation of isoflavonoid phytoalexins. *Acta Bot. Neerl.* **28**: 227–229.

—, F. W. DE VRIES, C. A. LANDHEER & A. VAN VELDHUIZEN (1980a): 3-Hydroxy-maackiainisoflavan, a pisatin metabolic produced by Fusarium oxysporum f. sp. pisi. *Phytochemistry* **19**: 000–000.

—, — & M. PLATERO SANZ (1980b): The mechanism of pisatin degradation by Fusarium oxysporum f. sp. pisi. *Physiol. Plant Path.* **16**: 119–133.

HEUVEL, J. VAN DEN, H. D. VAN ETTEN, J. W. SERUM, D. L. COFFEN & T. H. WILLIAMS (1974): Identification of 1a-hydroxy phaseollone, a phaseollin metabolite produced by Fusarium solani. *Phytochemistry* **13**: 1129–1131.

HIGGINS, V. J. (1975): Induced conversion of the phytoalexin maackiain to dihydromaackiain by the alfalfa pathogen Stemphylium botryosum. *Physiol. Plant Path.* **6**: 5–18.

—, A. STOESSL & M. C. HEATH (1974): Conversion of phaseollin to phaseollinisoflavan by Stemphylium botryosum. *Phytopathology* **64**: 105–107.

HOMANS, A. L. & A. FUCHS (1970): Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *J. Chromatog.* **51**: 327–329.

ITO, S., Y. FUJISE & A. MORI (1965): Absolute configuration of pterocarpinoids. *J. Chem. Soc. Chem. Comm.* 595–596.

KUROSAWA, K., W. D. OLLIS, B. T. REDMAN, I. O. SUTHERLAND, H. M. ALVES & O. R. GOTTLIEB (1978): Absolute configurations of isoflavans. *Phytochemistry* **17**: 1423–1426.

—, —, —, —, O. R. GOTTLIEB & H. MAGALHAES ALVES (1968): The absolute configurations of the animal metabolite, equol, three naturally occurring isoflavans, and one natural isoflavanquinone. *J. Chem. Soc. Chem. Comm.*: 1254–1267.

PACHLER, K. G. R. & W. G. E. UNDERWOOD (1967): A proton magnetic resonance study of some pterocarpan derivatives. The conformation of the 6a, 11a-dihydro-6H-benzofuro [3,2-c] [1] benzopyran ring system. *Tetrahedron* **23**: 1817–1826.

PERRIN, D. R. & I. A. M. CRUICKSHANK (1969): The antifungal activity of pterocarpans towards Monilinia fructicola. *Phytochemistry* **8**: 971–978.

SICHERER, C. A. X. G. F. & A. SICHERER-ROETMAN (1980): The R,S nomenclature of pterocarpan stereochemistry. *Phytochemistry* **19**: 485–486.

STEINER, P. W. & R. L. MILLAR (1974): Degradation of medicarpin and sativan by Stemphylium botryosum. *Phytopathology* **64**: 586.

VAN ETTEN, H. D. (1976): Antifungal activity of pterocarpans and other selected isoflavonoids. *Phytochemistry* **15**: 655–659.

— & S. G. PUEPPKE (1976): Isoflavonoid phytoalexins. In: *Biochemical Aspects of Plant-Parasite Relationships* (edited by J. FRIEND & D. R. THRELFALL), Academic Press, London, New York, San Francisco, p. 239–289.