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AUTONOMOUS AND INDUCED PTEROCARPANOID FORMATION IN THE LEGUMINOSAE

T. HIJWEGEN

Laboratory for Phytopathology, Agricultural University, Wageningen, The Netherlands

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Key Word Index—Lotoideae; Leguminosae; pterocarpanoid formation; phytoalexin production; isoflavanoid biosynthesis; chemotaxonomy.

Abstract—The occurrence of pterocarpanoids (either 'autonomously formed' or 'induced') is surveyed and a chemotaxonomic evaluation is made of this and new data. A hypothesis is proposed for their biosynthetic relationships involving a pterocarp-6a-en intermediate.

INTRODUCTION

DURING the past decade, pterocarpans and coumestones (6-oxo-pterocarp-6a-ens) have drawn much attention, the former as fungicides (phytoalexins),[1] the latter as estrogens.[2] These phytoalexins, are produced as a result of stress, of which fungal attack is probably the most extensively studied, and have concerned plant pathologists for many years. Their importance in disease resistance has aroused much dispute; evaluations range from their being considered responsible for almost all resistance[3] to being only one of many factors[4] or even of very doubtful significance.[5] Pterocarpans are generally accepted to be the phytoalexins of the Leguminosae.[1]

Some inconsistencies in our own experimental results[6] as well as in literature reports prompted us to survey the occurrence of pterocarpans among legumes.

RESULTS AND DISCUSSION

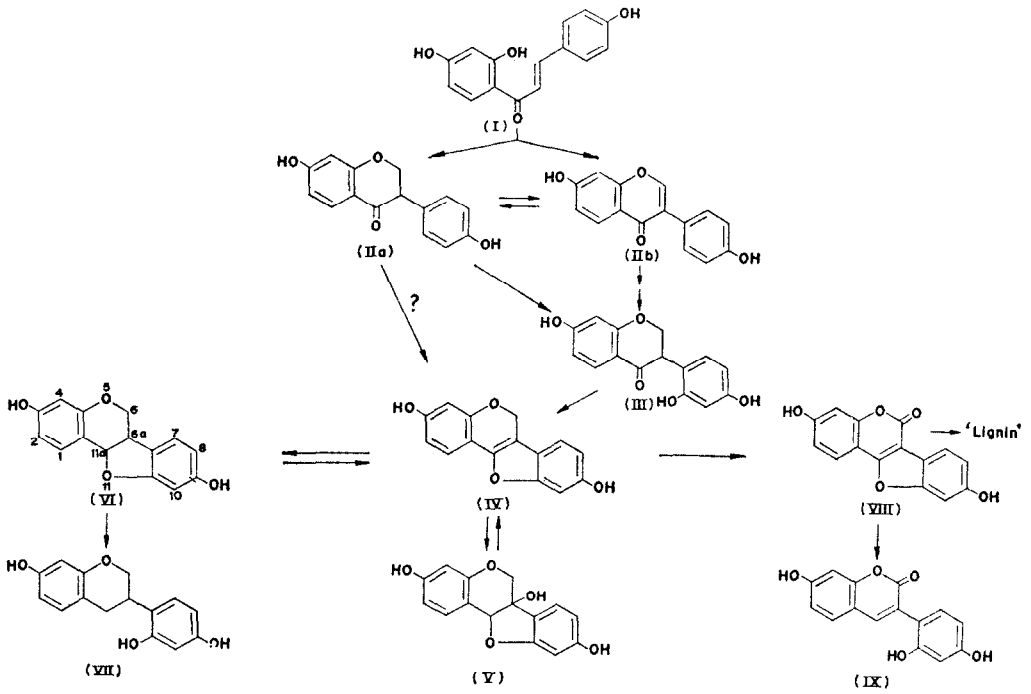
Biogenetic Considerations

Although some authors have drawn attention to possible interrelations between pterocarpans and coumestones,[7,8] no comprehensive scheme for their biogenesis has yet been proposed.

The biosynthesis of coumestrol in the root system of *Phaseolus aureus* Roxb. has been extensively investigated by Grisebach *et al.*[7,9] In the earlier publication,[7] two alternative pathways, one involving a 4-hydroxy-3-arylcoumarin and the second a pterocarp-6a-en intermediate, were proposed. More recently,[9] however, a 4-hydroxy-3-arylcoumarin was

[1] D. R. PERRIN and I. A. M. CRUICKSHANK, *Phytochem.* **8,** 971 (1969); and references therein.
[2] E. M. BICKOFF, R. R. SPENCER, S. C. WITT and B. E. KNUCKLES. *Studies on the Chemical and Biological Properties of Coumestrol and Related Compounds.* U.S. Dept. of Agriculture, Technical Bulletin No. 1408 (1969).
[3] I. A. M. CRUICKSHANK, *Ann. Rev. Phytopath.* **1,** 351 (1963).
[4] I. M. SMITH, *Physiol. Plant Path.* **1,** 85 (1971).
[5] W. H. FUCHS, *Meded. Landb. Hogesch. Gent.* **30,** 1336 (1965).
[6] T. HIJWEGEN, *Neth. J. Pl. Path.* To be published.
[7] P. M. DEWICK, W. BARZ and H. GRISEBACH, *Phytochem.* **9,** 775 (1970).
[8] D. M. X. DONNELLY and M. A. FITZGERALD, *Phytochem.* **10,** 3147 (1971).
[9] J. BERLIN, P. M. DEWICK, W. BARZ and H. GRISEBACH, *Phytochem.* **11,** 1689 (1972).

excluded on experimental grounds and further evidence given to support the pathway via a pterocarp-6a-en (IV) (Scheme 1). Keen *et al.*[10] derived a pathway for 6a-hydroxyphaseollin biosynthesis by also assuming a pterocarp-6a-en as an intermediate.



SCHEME 1. PROPOSED GENERAL PATHWAY OF THE BIOSYNTHESIS OF PTEROCARPANOIDS (simplified).

Although the objection may be made that the intermediate(IV) is unstable, it should be emphasized that the dimethyl analogue has been isolated from *Swartzia madagascariensis*[11] and *Dalbergia decipularis*[12] and that another pterocarp-6a-en is the main isoflavanoid in *Swartzia leiocalycina*.[8]

It is attractive to consider (IV) as an intermediate, since: (a) oxidation will give rise to coumestones (VIII); (b) reduction may provide pterocarpans (VI); and (c) addition of water produces pterocarpans of the pisatin-type (V). Further reduction of pterocarpans may give rise to isoflavans (VII) whereas reduction of coumestones may yield 3-arylcoumarins, (IX) as in *Neorautanenia*.[13] Thus, all pterocarpanoids can be derived from one type of intermediate. At the moment no definite choice can be made between formation of (IV) from a 2'-hydroxyisoflavanone (III) or by direct oxidative cyclization of an isoflavanone (IIa), as suggested by Wong.[14]

[10] N. T. KEEN, A. I. ZAKI and J. J. SIMS, *Phytochem.* **11**, 1031 (1972).
[11] S. H. HARPER, A. D. KEMP and W. G. E. UNDERWOOD, *Chem. Commun.* **309** (1965).
[12] R. DE ALENCAR, R. BRAZ FILHO and O. R. GOTTLIEB, *Phytochem.* **11**, 1517 (1972).
[13] C. VAN DER M. BRINK, W. NEL, G. J. H. RALL, J. C. WEITZ and K. G. R. PACHLER, *J. South Afr. Chem. Inst.* **19**, 23 (1966).
[14] E. WONG, in *Progress in the Chemistry of Organic Natural Products* (edited by W. HERZ, H. GRISEBACH and A. I. SCOTT), Vol. 18, p 57, Springer, Wien (1970).

TABLE 1. DISTRIBUTION OF PTEROCARPANOIDS IN THE LEGUMINOSAE

Taxon*	Production* autonomous aerial parts P	C	autonomous roots P	C	induced aerial parts P	C	induced roots P	C
CAESALPINOIDEAE								
Apuleia leiocarpa (Vog) Macbr.[32]	+							
LOTOIDEAE								
Swartzieae								
Swartzia leiocalycina[8]	+	+						
S. madagascariensis Desv.[11,41]	+	+						
Sophoreae								
Sophora spp.[1]	+							
Maackia amurensis[15]	+							
Pericopsis angolensis[41]	+							
Podalyrieae								
Baptisia australis[15]	−		+					
Genisteae								
d. Spartiinae								
Laburnum anagyroides Med.[33]	+	−	+	−				
Dalbergieae								
a. Pterocarpinae								
Baphia nitida Lodd.[27,28]	+							
Pterocarpus (5 spp.)[27,28,42]	+							
'*Dalbergieae pantropicales*' (10 out of 11 spp.[18])	−							
'*Dalbergieae brasilianae*' (6 spp.)[12,18]	+	+†						
Machaerium (7 out of 8 spp.)[18]	+							
b. Lonchocarpinae								
Lonchocarpus laxiflorus[29]			+					
c. Geoffraeinae								
Andira inermis (Wright) H.B.K.[15,26]	+							
Galegeae								
a. Indigoferinae								
Indigofera (2 spp.)[33]	+	−						
b. Psoraliinae								
Psoralea bituminosa L.[33]	+	+	−	+	+			
P. corylifolia L.[24]		+						
d. Tephrosiinae								
Wistaria sinensis DC[33]	+	−						
e. Robiniinae								
Robinia pseudoacacia L.[33]	+	−	+	−				
f. Coluteinae								
Colutea cruenta Ait.[33]	±	−	−	−				
g. Astragalinae								
Caragana arborescens Lam[33]	−	−	−	−	+	−		
Phaseoleae								
a. Glycininae								
Glycine max (L.) Merr[10,25]	−	−			+	+		
d. Diocleinae								
Canavalia virosa Wight and Arn.[4]	−				(+)			
C. ensiformis (L.) DC[4]	−				(+)			
f. Phaseolinae								
Lablab niger Medik.[4]	−				(+)			
Vigna sinensis (L.) Savi ex Hassk.[4]	−				(+)			
Neorautanenia edulis C.A. Sm.[34,43,44]			+	+				
N. ficifolia (Benth. ex Harv.) C.A. Sm.[13]			+	+				

TABLE 1—*continued*

Taxon*	Production* autonomous aerial parts P	C	roots P	C	induced aerial parts P	C	roots P	C
Pachyrrhizus erosus Urban[40]				+				
Phaseolus mungo L.[33]	−	−	−	+				
P. aureus Roxb.[7,33,39]	−	−	−	+				
P. coccineus L.[33]	−	−	−	+	+			
P. vulgaris L. [33,35,36]	−	−	−	+	+	+		
Trifolieae								
Trigonella foenum-graecum L.[33]	−	−	−	−	+	−	+	−
Medicago sativa L.[2,33,37,38]	−	−	−	+	+	+		
Melilotus albus Med.[33]	−	−	−	+	+		+	+
Trifolium spp.[28]		+						
T. pratense L.[16,19,33]	−	−	−	−	+	−	+	−
Loteae								
Lotus uliginosus Schk.[33]	−	−	−	−	+	−	+	−
Anthyllis vulneraria L.[33]	−	−	−	−	+	−	+	−
Vicieae								
Cicer arietinum L.[4]	(+)							
Pisum spp.[17]	−	−	−	−	+	−	+	−
Hedysareae								
a. Coronillinae								
Ornithopus sativus Brot.[33]	−	−	−	−	+	−	+	−
b. Euhedysarinae								
Onobrychis viciifolia Scop.[33]	−	−	−	−	+	−	+	−
e. Stylosanthinae								
Arachis hypogea L.[33]	−	−	−	−	+	−	+	−
f. Desmodiinae								
Desmodium gangeticum[22]	+							

* *Key.* Circumscription of tribes is according to Taubert[30] with the exception of Swartzieae which are included in Lotoideae; the sequence, however, is according to Hutchinson.[31] P—pterocarpan (reduced form), C—coumestone (oxidized form). + present, − absent, ± spectrophotometrically and chromatographically detectable, but less than 1 mg/kg fr. wt, blank spaces denote lack of data. (+) Data of Smith,[4] UV spectra so closely resembling phaseollin and medicarpin that it is assumed by the present author to be a pterocarpan.

† Only one of the species, *Dalbergia decipularis* Rizz. & Matt., has been reported to contain also 3,9-dimethoxy-6-oxo-pterocarp-6a-en.

[15] P. LEBRETON, K. R. MARKHAM, W. T. SWIFT, III, OUNG-BORAN and T. J. MABRY, *Phytochem.* **6,** 1675 (1967).
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[23] D. R. PERRIN, C. P. WHITTLE and T. J. BATTERHEM, *Tetrahedron Letters* **1673** (1972).
[24] H. N. KHASTGIR, P. C. DUTTAGUPTA and P. SENGUPTA, *Tetrahedron* **14,** 275 (1961).
[25] N. T. KEEN, *Physiol. Pl. Path.* **1,** 265 (1971).

Chemotaxonomic Evaluation

In Table 1 data on pterocarpanoid distribution derived from literature (mainly concerning tropical trees) or from our own results (mostly on herbaceous plants)[33] are summarized. There are two significant features, namely: (a) autonomous formation in most trees (the most primitive taxa) vs. regulated formation in most herbs, so that the accepted course of evolution from trees to herbaceous plants is clearly reflected in pterocarpanoid synthesis; and (b) formation of the oxidized form vs. the reduced form (compare results in the Phaseolinae with those in the Trifolieae). A phenomenon, which is not apparent from Table 1, is the increasing specialization effectuated in the decreasing number of compounds, e.g. from eight pterocarpans in *Swartzia madagascariensis* to only one in, e.g. *Baptisia australis*,[15] *Trifolium pratense*[16]* and *Pisum sativum*.[17]

Though at present no physiological function can be ascribed to the pterocarpanoids, their extreme conservatism is remarkable; their formation may be repressed, but the ability to synthesize these compounds in stress situations never seems to be lost, as far as can be judged from the limited experimental data. A special position is taken by the '*Dalbergieae pantropicales*', as already noted by Braga de Oliveira *et al.*[18] In these plants metabolic capacities seem to have shifted so far to neoflavanoid formation that pterocarpanoids are either absent or only trace amounts accumulate. It is not known whether these species could be induced to synthesize pterocarpanoids or not.

In general, when pterocarpanoid synthesis has been demonstrated, plants should be grown under aseptic conditions to see whether formation is autonomous or induced. This is especially true for roots, since these are always surrounded by soil micro-organisms. Of course, this can only be done easily with small herbaceous plants. The following species were grown under aseptic conditions by the present author: *Trigonella foenum-graecum*, *Melilotus albus*, *Medicago sativa*, *Trifolium pratense*, *Anthyllis vulneraria*, *Phaseolus vulgaris*, *P. aureus*, *P. mungo*, *Ornithopus sativus* and *Arachis hypogea* (Table 1). On the other hand, plants in which the presence of pterocarpanoids has not yet been established should be

* *Note.* Recently, however, it has been reported, that in *Trifolium pratense* two pterocarpans occur;[19] possibly there are differences between varieties.

[26] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
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[34] B. L. VAN DUUREN, *J. Org. Chem.* **26**, 5013 (1961).
[35] W. G. RATHMELL and D. S. BENDALL, *Physiol. Pl. Path.* **1**, 351 (1971); and references therein.
[36] D. R. PERRIN, *Tetrahedron Letters* **29** (1964).
[37] H. GRISEBACH and W. BARZ, *Z. Naturf.* **18b**, 466 (1963).
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[42] M. R. PARATHASARATHY, R. N. PURI and T. R. SESHADRI, *Indian J. Chem.* **7**, 118 (1969).
[43] G. J. H. RALL, J. P. ENGELBRECHT and A. J. BRINK, *Tetrahedron* **26**, 5007 (1970).
[44] G. J. H. RALL, A. J. BRINK and J. P. ENGELBRECHT, *J. South Afr. Chem. Inst.* **25**, 25 (1972); *Chem. Abs.* **76**, 124141k (1972).

examined to see if they accumulate substances of this kind under particular circumstances. In the case of autonomous formation, it is difficult to assess whether production is increased by stress. In *Psoralea bituminosa* leaves however, it was clearly demonstrated by us that pterocarpanoid accumulation was greatly enhanced by treatment with mercuric chloride.

It has been suggested that regulation of pterocarpanoid synthesis may be at the isoflavone level.[20] This has in fact been demonstrated for coumestrol formation in *Phaseolus aureus* roots.[7] Also, in our experiments with *Trifolium pratense*, trifolirhizin formation was always accomplished at the expense of isoflavones. However, a different situation apparently holds in the case of *Pisum* roots. Extracts of healthy roots lacked isoflavanoids, the UV spectrum only revealing small amounts of a substance with spectral characteristics of coniferyl alcohol; no isoflavones could be detected. After fungal infection, however, pisatin formation could readily be observed.

Considering the specificity of most plant-pathogen combinations and the apparent limited number of effective pterocarpans as fungitoxic compounds,[1] the overall pattern of distribution in the Leguminosae (Table 1) does not strengthen our confidence that these compounds are of major importance in plant resistance.

From the Limited data available (Table 1) it is clear that pterocarpan and coumestone formation is characteristic for the subfamily Lotoideae of the Leguminosae; there is only one report in another family, viz. of wedelolactone and desmethylwedelolactone in the Compositae.[21] Except for three recently discovered members,[8,22] all compounds seem to be derived from 5-deoxyisoflavones. They are apparently of little value for differentiating between the lower taxonomic categories, except that the *Lonchocarpus* pterocarpans are distinct in having oxygen functions at positions 3, 7, 9 and 10, corresponding to those of rotenone.[29]

Most abundant are 3-hydroxylated pterocarpans or their glycosides (tribes Swartzieae, Sophoreae, Podalyrieae, Genisteae, Trifolieae, some Galegeae, Dalbergieae, Phaseoleae) followed by 3-methoxy pterocarpans (tribes Swartzieae, Dalbergieae-subtribe Pterocarpinae, Vicieae genus *Pisum*). Isoprenylated and furano-compounds derived from them are rare, being confined to the Phaseoleae, *Desmodium*, *Psoralea* and to the only species with pterocarpanoids belonging to the subfamily Caesalpinoideae. One of the two isoprenyl residues in gangetin from *Desmodium gangeticum*[22] as well as the isoprenyl residues in phaseollidin from *Phaseolus vulgaris*[23] and psoralidin from *Psoralea corylifolia*[24] no longer form part of a ring structure. As possession of isoprenyl groups is a dispensable character,[26] this might be considered as a loss mutation.

6a-Hydroxylated compounds are very rare. Only three have been reported so far, viz. pisatin from *Pisum* species,[17] 6a-hydroxyphaseollin from soybean, *Glycine max*,[25] and variabilin from *Dalbergia variabilis*.[27] They are, however, so randomly distributed that they have no chemotaxonomic value. The high score of coumestone derivatives in the root systems of plants belonging to the Phaseolinae is especially noteworthy; it might be characteristic for the whole subtribe or even a larger group. More extensive investigations will certainly demonstrate whether this assumption is correct.