Phytochemistry, 1980, Vol. 19, pp. 2359–2365. Pergamon Press Ltd. Printed in England.

A CHEMICAL DICHOTOMY IN PHYTOALEXIN INDUCTION WITHIN THE TRIBE VICIEAE OF THE LEGUMINOSAE

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(*Received* 21 *March* 1980)

Key Word Index—*Lathyrus*; *Lens*; *Pisum*; *Vicia*; Leguminosae; phytoalexins; pterocarpans; furanoacetylenes; pisatin; wyerone; chemotaxonomy.

Abstract—Using either leaf or cotyledon tissues, the phytoalexin response of over 60 representative members of the genera *Lathyrus*, *Lens*, *Pisum* and *Vicia* was determined. Twenty-nine *Vicia* species produced furanoacetylenes, as did the two *Lens* species examined. By contrast, *Pisum* and *Lathyrus* failed to produce any of these phytoalexins, but formed pisatin instead. Thus, pisatin was formed as the major phytoalexin in 29 of 31 *Lathyrus* species tested, representing 10 sections within the genus. This dichotomy in phytoalexin response is discussed in relation to the taxonomy of the tribe Vicieae.

INTRODUCTION

Phytoalexins are fungitoxic substances, produced *de novo* in plants around the site of infection following microbial invasion. They have been extensively studied in a variety of plant–parasite interactions, because of their possible role in disease resistance [1, 2]. A taxonomic element is present in phytoalexin synthesis in that the type of compound formed during the interaction may vary from plant to plant and from family to family. The actual use of phytoalexin induction as a new dynamic approach to taxonomic research was first proposed quite recently from experiments in the genus *Trigonella* (Leguminosae) [3]. Here it was found possible to divide the 35 species studied into three main groupings on the basis of different phytoalexin responses. Subsequent studies in a range of other legume genera have confirmed the utility of this new approach to systematic problems at least within the Leguminosae [4].

In the present work, we were interested in examining the potential of the phytoalexin approach to generic classification within a tribe. For this purpose, we chose the tribe Vicieae, economically an important group and one in which some significant information was already available on phytoalexin production. Two very different responses were already known within the tribe. Thus the pea *Pisum sativum* is known to produce the pterocarpan pisatin [5], whereas the broad bean *Vicia faba* yields several different furanoacetylene phytoalexins [6]. Our choice of the Vicieae was also partly determined, because taxonomic and phyletic relationships within the tribe are still a matter of debate. Also much chemical work has already been carried out on many of the plants in this tribe [7].

The tribe Vicieae, one of the most advanced groups within the subfamily Papilionoideae, comprises four main genera: *Lathyrus*, *Lens*, *Pisum* and *Vicia*. Two other genera, *Abrus* and *Cicer*, formerly included within the Vicieae, have been separated off as belonging to monotypic tribes [8]. Our main task, therefore, was to survey representative members of these four genera for their phytoalexins and the present paper records these results in detail. Some of our results have already been published briefly in preliminary form [9, 10].

RESULTS

The genus Pisum

Although many specific epithets have been employed to describe *Pisum* species, only two taxa are now recognised in the genus: the mainly cultivated *P. sativum* L. and the wild *P. fulvum* Sibth. et Sm. [11]. The phytoalexin response was extensively studied in pea material by Cruickshank and Perrin [12] who were the first to identify the pterocarpan pisatin (**1**) as the characteristic phytoalexin of these plants. More recently, Stoessl [13] has identified the closely related maackiain (**2**) as a minor component of the phytoalexin response in *P. sativum*.

Working with a range of cultivated and wild *Pisum* accessions, we have completely confirmed these earlier results. Further, we can report that *P. fulvum* is similar to *P. sativum* in producing maackiain as a minor phytoalexin. The ratio of the two components in both species is approximately the same, pisatin being produced in 20 times the concentration of that of maackiain. Actual pisatin: maackiain ratios obtained, after inoculation of leaflets with a spore suspension of *Helminthosporium carbonum* were: *P. sativum* cv Meteor leaf diffusate 22.3:1, within the leaf 19.2:1; *P. fulvum* leaf diffusate 19.5:1, within the leaf 18.3:1.

The genus Lathyrus

Lathyrus is a large genus of perennial and annual herbs and contains 144 species within 13 sections [14]. Thirty-one species grown from seed were tested for their phytoalexin response (Table 1). Pisatin was found to be the major phytoalexin: it was detected as the only phytoalexin in 27 accessions of 16 spp., as the major phytoalexin in 14 accessions representing 11 spp. and as a minor phytoalexin in a further 2 spp.

Table 1. Phytoalexins of *Lathyrus* species

Species	Phytoalexins produced Pi	Mk	Va	Md	Sources
Section *Orobus* (Old World Members)					
Lathyrus davidii Hance	+	—	(+)	—	PAS
L. japonicus Willd.	+	—	—	—	HA
L. linifolius (Reichard) Bässler	(+)	(+)	(+)	(+)	AM
L. niger (L.) Bernh.	(+)	—	—	—	AM
L. niger (L.) Bernh.	+	—	—	—	OX
L. palustris L.	(+)	+	—	—	GO
L. pisiformis L.	+	—	—	—	VP, BR
L. vernus (L.) Bernh.	—	—	+	—	RE
Section *Orobus* (New World Members)					
L. ochroleucus Hooker	+	—	(+)	—	AL
Section *Lathyrostylis*					
L. pannonicus (Jacq.) Garcke	+	—	—	—	UN
Section *Lathyrus*					
L. amphicarpos L.	+	—	—	—	CO
L. annuus L.	+	—	(+)	—	BA, NO
L. cicera L.	+	—	—	—	VA
L. cicera L.	+	(+)	—	—	CO
L. grandiflorus Sibth. & Sm.	+	(+)	—	(+)	MN
L. hirsutus L.*	+	(+)	(+)	(+)	CP
L. hirsutus L.*	+	—	+	—	GA
L. hirsutus L.*	+	—	—	+	BR
L. latifolius L.	+	—	—	—	MO
L. rotundifolius Willd.	+	—	—	—	PO
L. odoratus L.*	+	—	(+)	—	cv Air Warden, Johnson's Giant Wave, Noel Sutton, JA, MP
L. odoratus L.*	+	—	—	—	cv Frolic, Swan Lake, BE, BU
L. sativus L.	+	—	—	—	CP
L. sylvestris L.	+	+	(+)	—	AM
L. tingitanus L.	+	—	—	—	FR
L. tuberosus L.	(+)	—	(+)	+	FR
L. tuberosus L.	+	+	(+)	—	JA
L. tuberosus L.	+	+	—	—	HA
Section *Orobon*					
L. roseus Steven	(+)	(+)	—	—	FB
Section *Pratensis*					
L. laxiflorus (Desf.) Ktze.	+	—	—	—	KW, HER
L. pratensis L.	(+)	—	—	—	OX, WH
Section *Aphaca*					
L. aphaca L.	+	(+)	—	—	CO, JA
Section *Clymenum*					
L. clymenum L.	+	—	—	—	BD, MO
L. ochrus (L.) DC.	+	(+)	—	—	CO, MO
Section *Orobastrum*					
L. setifolius L.	+	—	—	—	MO, PI
Section *Linearicarpus*					
L. angulatus L.	+	—	—	—	CO
Section *Nissolia*					
L. nissolia L.†	—	—	—	+	OX, FR, NA
Section *Notolathyrus*					
L. magellanicus Lam.	+	—	—	—	MU

Key: Pi = pisatin; Mk = maackiain; Va = variabilin; Md = medicarpin; + = present; — = not detected; (+) = trace. For key to sources, see under Table 2. Sectional classification after Kupicha [14].

* Produces chromones as well (see text).

† Produces pterocarpans, nissolin and methylnissolin (see text).

Three other pterocarpans, maackiain (**2**), variabilin (**3**) and medicarpin (**4**) were also variously detected as phytoalexins in *Lathyrus*. Variabilin was noted in nine species and, with the exception of *L. vernus*, it always co-occurred with pisatin. Maackiain was found in ten species, all of which already produced pisatin, while medicarpin was found in only five species, usually as a trace constituent accompanying maackiain (Table 1). Medicarpin is particularly widely distributed in the Papilionoideae, especially in members of the tribe Trifolieae [2, 4], so its presence in *Lathyrus* is not unexpected.

The sweet pea *Lathyrus odoratus* was unusual in its phytoalexin response, since it yielded two novel chromones besides the pterocarpans pisatin and variabilin. Structural studies on these chromones, lathodoratin and methyl-lathodoratin, are in progress. It is, however, worth reporting that lathodoratin occurs in one other *Lathyrus* species studied, in all three accessions examined of *L. hirsutus*. This species is not only in the same section of the genus but also seems to be particularly close morphologically to *L. odoratus*, since it is the only annual species which will hybridize reciprocally with it [15].

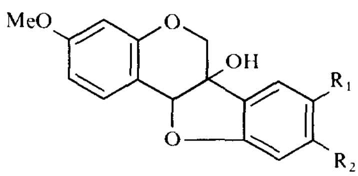
An anomalous phytoalexin response was also found in *L. nissolia*, the only member of section *Nissolia*, and a very distinctive taxon with its linear grass-like phyllodes which replace the more usual leaflets and tendrils found in other species. It is the only species, apart from *L. vernus*, where pisatin is not detectable. Instead, two novel pterocarpans, nissolin (**5**) and methylnissolin (**6**) are produced in quantity [16], together with smaller amounts of medicarpin. The novel phytoalexin chemistry thus revealed in *L. nissolia* fits in well with its isolated position within the genus.

In the case of 13 species, it was possible to examine more than one accession of seed and while there were quantitative differences in phytoalexin response, qualitative variations were usually absent. Significant variation was, however, noted in the case of *L. tuberosus*, three accessions of which were examined (Table 1). Of the four phytoalexins detected in this species, pisatin was the only one produced regularly by all three accessions. Such variation might have been due to botanical misidentification, and yet the plants all appeared to fit the description for this species. In addition, when the leaf flavonoids were examined, all three accessions showed virtually the same pattern. Thus, the three accessions appear to belong to the same species, since intraspecific variation in leaf flavonoids is well marked in the genus as a whole (R. Grayer-Barkmeijer, personal communication).

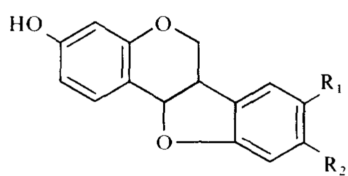
The genus Vicia

Vicia is a large genus of 140 species, which are classified into 21 sections [17]. It is close to *Lathyrus* and some species appear to be intermediate and are difficult to assign definitively to either genus [18]. Phytoalexins have been examined so far in only three species. The furanoacetylene wyerone (**7**) is known to be produced as a phytoalexin in *Vicia faba* [19], *V. narbonensis* and *V. galilea* [6]. Other related furanoacetylenes, including wyerone acid and wyerone epoxide (**8**) are also formed [20] in *V. faba*. Furthermore, in this plant, trace amounts of medicarpin are detectable, so that the production of pterocarpan, such a characteristic feature of the legume phytoalexin response, is not completely absent from *Vicia*.

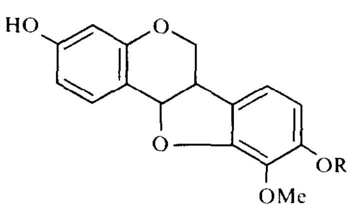
In the present survey, wyerone was detected as a phytoalexin in 28 species, while wyerone epoxide generally



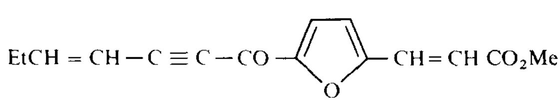
Pisatin, **1** $R_1 = R_2 = OCH_2O$
Variabilin, **3** $R_1 = H$, $R_2 = OMe$



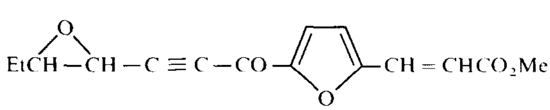
Maackiain, **2** $R_1 = R_2 = OCH_2O$
Medicarpin, **4** $R_1 = H$, $R_2 = OMe$



Nissolin, **5** R = H
Methylnissolin, **6** R = Me



Wyerone **7**



Wyerone epoxide **8**

accompanied it in 15 of these species. In only one species, *V. articulata*, and in one accession of another species, *V. ervilia*, did wyerone epoxide occur in the absence of wyerone (Table 2). In none of the 29 species surveyed did pterocarpan or isoflavan phytoalexins occur to any detectable extent.

The 29 species of *Vicia* surveyed (Table 2) belong variously to 15 sections within the genus and include those sections considered to be the most primitive (*Vicilla*) and the most advanced (*Peregrinae*) [17]. Also the species examined are from both the Old and New Worlds and include plants with both perennial and annual habit. The survey was thus reasonably representative of *Vicia*, so that it would appear that the ability to produce furanoacetylenes is a characteristic of the whole genus.

The genus Lens

Lens is a small genus, consisting of the cultivated crop plant *L. culinaris* together with four wild species [21]. Only *L. culinaris* and *L. nigricans* were available for phytoalexin induction experiments. Both responded in the same way, giving major amounts of the pterocarpan, variabilin, detected earlier in *Lathyrus* (see above). However, the two furanoacetylene phytoalexins wyerone and wyerone epoxide were also readily detected and then fully characterized as part of the phytoalexin response. Of the two, wyerone epoxide was the more abundant; this is the reversal of the normal situation in *Vicia* [6].

DISCUSSION

The results reported in this paper show for the first time that it is possible to obtain a phytoalexin response uniformly throughout a tribe within the family Leguminosae. Practically every species tested gave a positive response when challenged with *Helminthosporium carbonum* or *Botrytis cinerea* spores. Furthermore, there are significant variations in pattern to make such a project useful from the taxonomic viewpoint. The data obtained, summarized in Table 3, are of systematic interest variously at the tribal, generic and specific levels of classification.

At the tribal level, it may be noted that the pattern of phytoalexins produced on fungal induction sets the Vicieae apart from any other tribe within the Leguminosae. In particular, the furanoacetylene phytoalexins of *Lens* and

Table 2. Phytoalexins in *Vicia* species

Species	Phytoalexin WY	Phytoalexin WE	Source
Subgenus *Vicilla*			
Section *Vicilla*			
Vicia unijuga A. Braun	+	(+)	BD
V. pisiformis L.	+	−	MA
V. dumetorum L.	+	−	FR
Section *Cassubicae*			
V. cassubica L.	+	−	MA
V. orobus DC.	+	+	MA
Section *Cracca*			
Old World Member, Perennial			
V. cracca L.	+	(+)	KW
Old World Members, Annuals			
V. villosa Roth.	+	(+)	MA
V. disperma DC.	+	(+)	LO
New World Member			
V. floridana S. Wats.	+	−	CRG
Section *Pedunculatae*			
V. onobrychioides L.	+	−	AT
Section *Americanae*			
V. americana Muhl. ex Willd.	+	−	LI
Section *Volutae*			
V. biennis L.	+	−	JBH
Section *Panduratae*			
V. cretica Boiss. & Heldr.	+	−	UN
Section *Ervoides*			
V. articulata Hornem.	−	+	CRG
Section *Ervilia*			
V. ervilia (L.) Willd.	(+)	+	FR
V. ervilia (L.) Willd.	−	+	MA
Section *Australes*			
V. graminea Smith	+	−	GA

Table 2. (*Continued*)

Species	Phytoalexin WY	WE	Source
Subgenus *Vicia*			
Section *Atossa*			
V. sepium L.	+	+	HE
Section *Vicia*			
V. sativa L.	+	+	KW, FR, GI
V. sativa L.	+	—	ER
V. grandiflora Scop.*	+	+	CP
V. lathyroides L.	+	—	MO
Section *Faba*			
V. faba L.	+	+	MA, cv The Sutton
V. narbonensis L.	+	+	GT
V. bithynica (L.) L.	+	—	UN
Section *Hypechusa*			
V. hybrida L.	+	+	KW
V. hyrcanica Fisch. & Meyer	+	—	MO
V. lutea L.	+	—	PI
V. pannonica Crantz	+	—	TAP
Section *Peregrinae*			
V. michauxii Spreng.	+	+	MO
V. peregrina L.	+	+	FA

Key: WY = wyerone; WE = wyerone epoxide; + = present; — = not detected; (+) = trace.

Key to sources of accessions of *Lathyrus* and *Vicia* species: PAS = BG (Botanic Garden), Principal Academy of Sciences, Moscow, USSR; HA = BG, Hamburg; AM = BG, Amsterdam; OX = BG, University of Oxford; GO = BG, Gothenberg; UP = BG, University of Uppsala; BR = BG, Brussels; RE = BG, University of Reading; AL = Devonian BG, University of Alberta, Canada; UN = unknown; CO = BG, Coimbra, Portugal; BA = BG, Barcelona; NO = John Innes Institute, Norwich; VA = BG, Vacratot, Hungary; MN = BG, Munich; CP = BG, Copenhagen; GA = Institut für Kulturpflanzenforschung Gatersleben; PO = BG, University, Posnan; JA = BG, Nikit, Jalta, USSR; BE = National BG, Belgium; BU = Budapest Fovaros, Budapest; MP = Jardin des Plantes, Montpellier; FR = BG, University of Frankfurt-am-Main; FB = BG, University of Freiburg; KW = The Royal BG, Kew; HER = Mrs. Souster, The Herbarium, University of Reading; WH = Whiteknights, Reading; BD = BG, Berlin-Dahlem; MO = BG, Moscow; NA = BG, Nantes; MA = BG, Johannes Gutenberg University, Maintz; LO = University of London Botanical Supply Unit; CRG = Dr. C. R. Gunn, USDA, Maryland; AT = BG, University of Athens; LI = BG, Botanical Academy of Sciences, Kaunas, Lithuania; JBH = J. B. Harborne, University of Reading; HE = BG, University of Helsinki; GI = BG, Giessen; ER = BG, Academy of Sciences, Erevan, USSR; GT = BG, Gottingen; PI = Orto Botanico, Pisano; TAP = Agrobotanic Institute, Tapioszele, Hungary; FA = BG, University of Karlsruhe.

Table 3. Phytoalexin variation within the tribe Vicieae

Genus	No. of spp. surveyed	Furanoacetylenes	Pterocarpans
Lens	2	Wyerone, wyerone epoxide	variabilin
Vicia	29	Wyerone, wyerone epoxide in 28/29 spp.	medicarpin in *V. faba* only
Pisum	2	None	pisatin
*Lathyrus**	31	None	pisatin in 29/31 spp.; maackiain, variabilin and medicarpin occasional; nissolin, methyl-nissolin in *L. nissolia*

* Chromones found in *L. odoratus* and *L. hirsutus* in addition to pterocarpan derivatives.

Vicia are peculiar to the Vicieae and have not been recorded elsewhere, in spite of a very wide survey within the family [4]. Also the 6a-hydroxypterocarpan pisatin, which is the dominant phytoalexin produced throughout *Lathyrus* and *Pisum*, is characteristic of the group and is not found elsewhere as a major component of the phytoalexin reaction. Pisatin has occasionally been recorded elsewhere in the Papilionoideae, but as a minor component of phytoalexin mixtures which mainly contain medicarpin and/or maackiain. As such, it has been detected in species of *Baptisia* and *Thermopsis* (both Thermopsideae) [4] and of *Caragana* (Galegeae) [22]. The absence of pisatin from *Cicer*, formerly included in the Vicieae, is taxonomically significant since this absence supports the decision to exclude *Cicer* from the tribe, based essentially on strong morphological and serological arguments [8]. As Ingham [23] has shown, *Cicer arietinum* produces a mixture of medicarpin and maackiain, two phytoalexins regularly produced in most genera of the Trifolieae.

The most important result to emerge from the present studies, however, is the striking dichotomy revealed in the Vicieae between *Vicia* and *Lens* on the one hand and *Pisum* and *Lathyrus* on the other. The former pair of genera are clearly distinguished from the latter in their additional ability to synthesize furanoacetylenes in response to fungal infection (Table 3). The fact that *Vicia* and *Lens* both apparently retain the ability to synthesize pterocarpan phytoalexins suggests that this is a basic phytoalexin response throughout the tribe. It can then be suggested that furanoacetylene synthesis has been superimposed on this basic pattern and largely replaces it in *Lens* and *Vicia*, perhaps in response to coevolution with a particularly damaging set of fungal parasites [2]. The relative toxicities of the two types of phytoalexin, however, remain to be compared in detail.

This chemical alignment of *Lens* with *Vicia* is reasonably acceptable taxonomically in view of the close morphological links and the fact that *Lens* was once included as a section within *Vicia*. The chemical separation of *Vicia* from *Lathyrus* on the basis of phytoalexin response is less in harmony with morphology and anatomy, since these two genera are generally regarded as being very close. It may be noted, however, that *Lathyrus* and *Vicia* also differ in the non-protein amino acid complements of the seeds [24]. The presence of these clearcut chemical differences between the two genera may in fact help in making a taxonomic decision about taxa which are morphologically intermediate in character.

Finally, it is clear from Tables 1 and 2 that phytoalexin induction is of systematic interest at the specific level within members of the Vicieae. It is satisfying chemotaxonomically that the morphologically distinctive grass-like *Lathyrus nissolia* is chemically distinguished in its phytoalexin response, producing two pterocarpans, nissolin and methylnissolin, not formed anywhere else in the genus. Even more interesting is the chemical distinctiveness of *L. odoratus* and *L. hirsutus* in synthesizing chromone phytoalexins. This is a type of phytoalexin not known elsewhere in the Leguminosae. It appears to be a significant finding and may warrant a taxonomic reassessment of the sectional position of these two *Lathyrus* species within the genus. The relative simplicity of the chromone nucleus, as compared to the isoflavonoid phytoalexins, as a fungitoxic material is also of note and the study of these phytoalexins is worthy of further exploration.

EXPERIMENTAL

Plant material. Plants were grown to maturity from seed. Every effort was made to obtain authenticated seed and, where possible, that collected in the field. The sources of seed samples are shown in Table 2. Vouchers of a representative selection of the plants are deposited in the University of Reading Herbarium.

Induction, isolation and identification of phytoalexins. Phytoalexins were induced in *Pisum*, *Lens* and *Lathyrus* species using the drop-diffusate technique with *Helminthosporium carbonum* as previously described [2]. *Vicia* and *Lens* species were examined for phytoalexins with cotyledons or seeds using *Botrytis cinerea* and a modification of the technique of Keen [25] as described earlier [10]. This was adopted for simplicity since acetylenic phytoalexins apparently do not accumulate in inoculation droplets in the same way as the isoflavonoid constituents, but remained mainly within the leaf. In order to check that the two techniques gave comparable results, the cotyledon method was used with *Pisum sativum* and *Lathyrus odoratus*; the results were essentially the same as those obtained in the drop-diffusate approach. In the experiments with *Vicia*, it is possible that wyerone acid was formed with wyerone and wyerone epoxide and a sample of this acid was obtained from the phytoalexin response in *Vicia faba* [6]. However, it appeared to be more labile than the other two wyerone derivatives, so no attempt was made to record its presence/absence in the different wild species.

Isolation and purification of the phytoalexins using TLC, and identification from TLC, UV, NMR and MS data are described elsewhere [9, 10, 16, 26].

Acknowledgements—The authors are very grateful to Dr. J. L. Ingham, University of Reading, and Dr. F. K. Kupicha, University of Cambridge, for valuable discussions and assistance. They also thank the SRC and ICI Plant Protection Ltd. for financial support.

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NOTE ADDED IN PROOF

Identification of the chromone phytoalexins of *L. odoratus* is reported in Robeson, D. J., Ingham, J. L. and Harborne, J. B. (1980) *Phytochemistry* **19**, 2171.