

BIOSYNTHESIS OF ISOFLAVONOID AND RELATED PHYTOALEXINS

Muhammad Afzal* and Galib Al-Oriquat

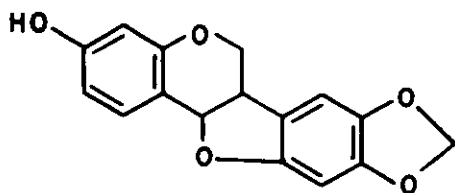
Biochemistry Department, Kuwait University

Kuwait

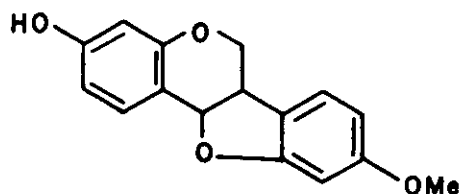
Abstract: Biosynthesis of isoflavonoid phytoalexins and related compounds is reviewed.

Low molecular weight antibiotics "phytoalexins" are produced following interactions between hypersensitive plant tissues and various parasites¹⁻³. These antibiotics may inhibit the growth of microorganisms pathogenic to the plant. This has suggested that phytoalexins may play an important role in plant disease resistance⁴⁻⁹. Reports contrary to this have also appeared claiming that phytoalexins have no role in plant defense mechanisms against fungal infection¹⁰. Phytoalexins are produced in the first few hours of the penetration of the parasitic cells into the invaded tissues¹¹⁻¹³. Invasions of the plant by viruses^{12,14} or bacteria¹⁵⁻¹⁸ also elicit phytoalexins.

The production of these compounds may also be triggered by abiotic treatment¹⁹⁻³⁰ of the plant by various factors such as heavy metals³¹⁻⁵³, polysaccharides⁵⁴⁻⁸⁸, peptides/proteins⁸⁷⁻⁹⁴, glycoproteins⁹⁵⁻¹⁰⁷, metabolic inhibitors^{31,108}, plant growth substances^{109,110}, oxidizing reducing agents¹⁰⁸, antimetabolites¹¹¹, DNA interchelating agents¹¹², RNA synthesis inhibitors¹¹²⁻¹¹⁴, irradiation with ultraviolet light¹¹⁵⁻¹¹⁹, mechanical injury^{120,121} and many other factors¹²²⁻¹²³. Biotic and abiotic elicitors act through different mechanisms^{134,135}. Thus fenugreek (*Trigonella foenum-graecum*) leaves on fungal infection produce maakian (1) and



(1)

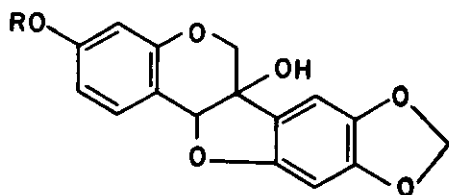


(2)

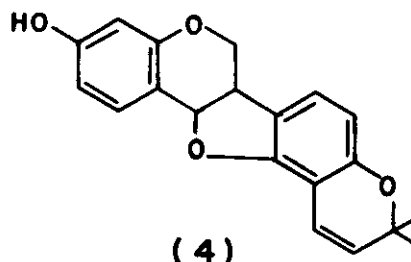
medicarpin (2) in equal amounts, but CuCl_2 and UV-treated seedlings produce only maackiain (1) and no medicarpin (2). However, Moesta et al.³⁶ and Bailey¹²¹ have concluded that biotic and abiotic elicitors act through the same mechanism. The genetic information for phytoalexin production is carried by the host and elicitors of such production, whether specific or non-specific, act through the host genome. Thus a given species generally produces the same phytoalexins irrespective of the challenging agent.

After two decades of research over 125 different phytoalexins ranging from isoflavoids¹³⁷⁻¹³⁹, terpenoids^{140,141}, isocoumarins^{142,143}, to polyacetylenic^{144,146}, in nature have been isolated and characterized from twelve families of plants. The majority of phytoalexins are produced by members of the Leguminosae and Solanaceae families of plants. In this area, widespread as it is, different views have been presented regarding phytoalexin biosynthesis, particularly of isoflavones. Understanding the biosynthetic pathways to these compounds is important in order to clarify defense mechanism of the plants.

Phenylalanine ammonia-lyase (PAL) is considered a key enzyme in flavonoid biosynthesis¹⁴⁷. Hadwiger and his co-workers have correlated¹¹⁸ PAL activity with phytoalexin production in excised pea and bean pod tissue. In pea tissue, the pea pathogen *Fusarium solani* f. sp. *Pisi* and bean pathogen *Fusarium solani* f. sp. *Phaseoli* are shown to be comparable in their abilities to stimulate the PAL activity¹⁴⁸, which is an intermediate enzyme in the production of pisatin^{149,150} (3a), phaseolin^{151,152} (4) and other structurally related phytoalexins¹⁵³. Increased level of pisatin (3a) and PAL activity in *Pisum sativum* treated with antihistaminic, antiviral, antimalarial and



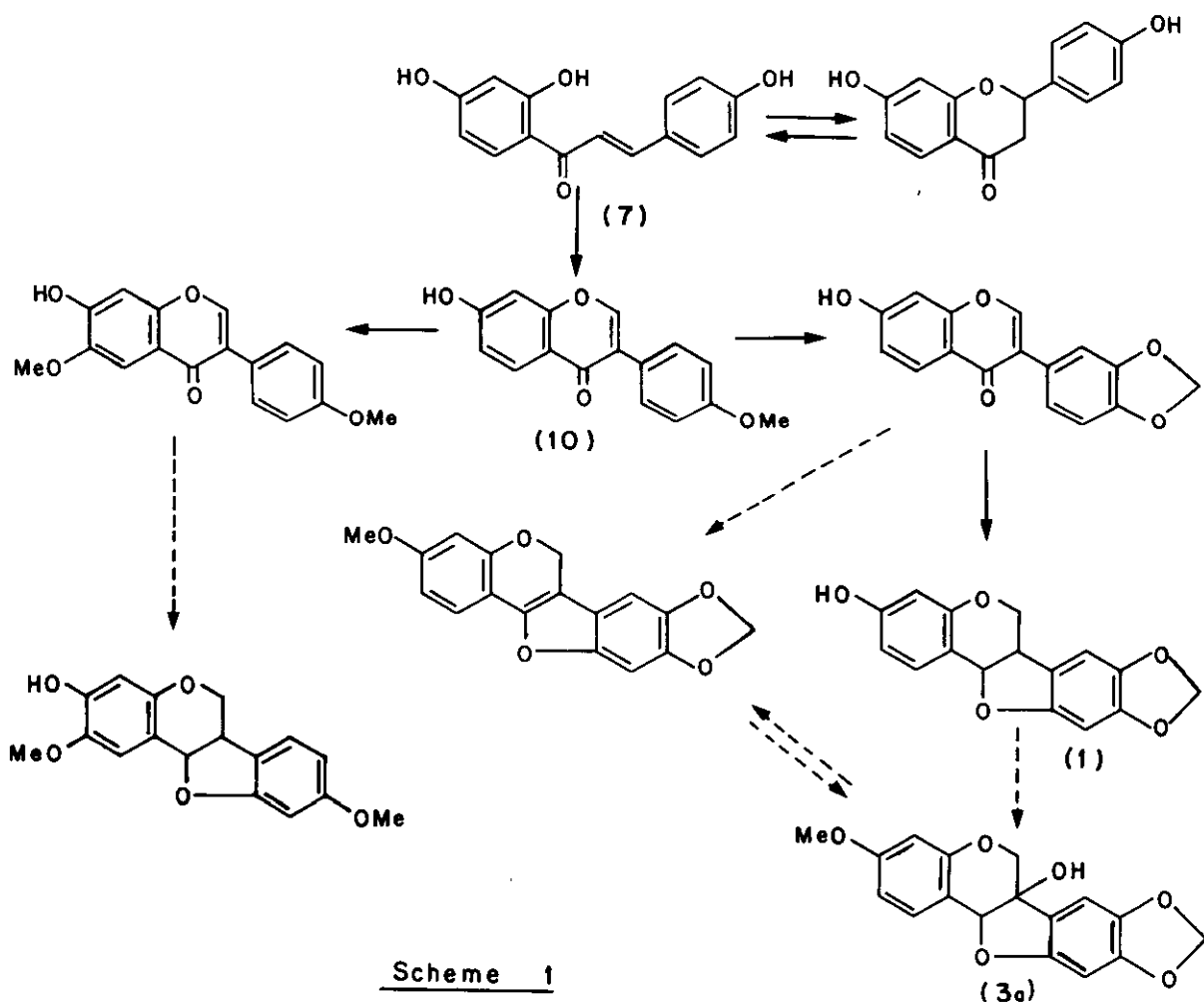
(3a): R = Me
(3b): R = H



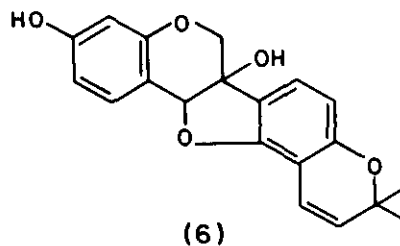
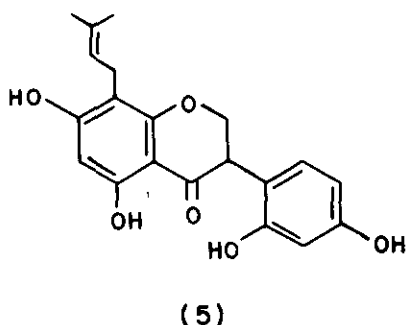
(4)

tranquilizing agents has also been reported¹⁵⁴. Changes in PAL activity have been frequently observed in host-pathogen interactions^{35,155,156} and in response to light¹⁵⁷, chemical¹⁵⁵ induction, wounding^{158,159} and stress conditions in various plants¹⁶⁰⁻¹⁶⁴.

While there are many reports that flavonoid phytoalexins production is associated with enhanced levels of PAL activity, there are also reports of phytoalexins synthesis under conditions in which PAL levels are depressed or equivalent compared with controls, suggesting little or no role of PAL in phytoalexin biosynthesis under certain conditions. However Creasy and Zucker¹⁶⁵ have reported that if the concentration of phenylalanine in a tissue remains constant then increases in PAL activity could still be involved in regulating phenylpropanoid levels. *Pisum sativum* accumulates five phytoalexins¹⁶⁶⁻¹⁶⁸ and innermin (3b) has been suggested¹⁶⁷ to be a precursor of pisatin (3a). Biosynthesis of pisatin and other flavonoid phytoalexins in *Pisum sativum* has been suggested by Carlson and Dolphin¹⁶⁹, as in Scheme 1.



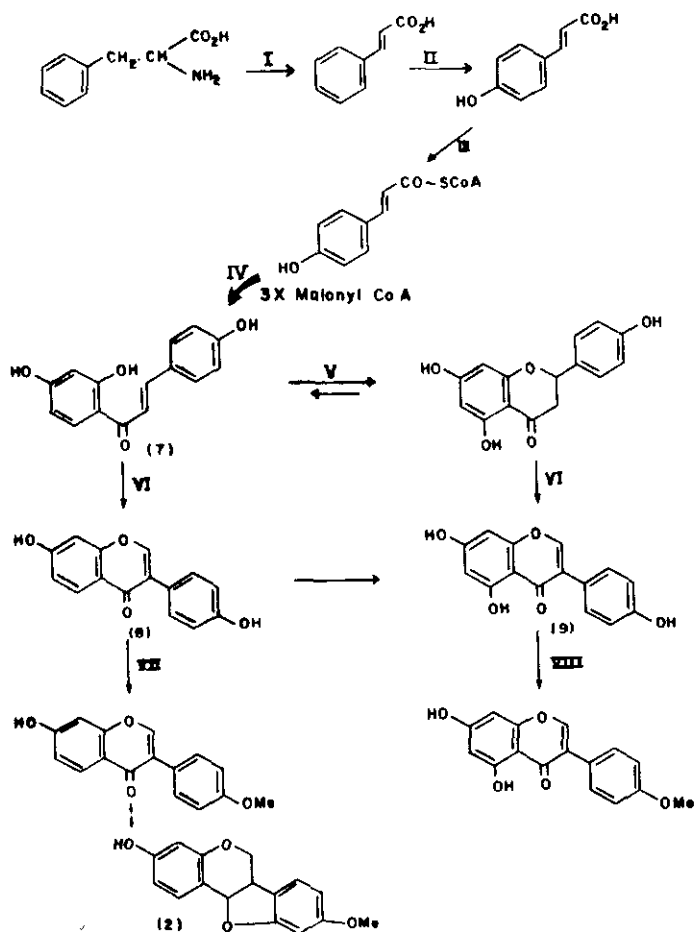
Although PAL is reported to catalyse the first step in the synthesis of medicarpin¹⁷⁰ (2), it has been suggested that its stimulation may not be a key step in regulation of medicarpin biosynthesis. Similarly Dixon and Fuller¹⁷¹ have shown that suspension cultures of Phaseolus vulgaris produced phaseolin (4) in the absence of added inducers and PAL activity was higher in control than in induced cultures. These workers have suggested that PAL was unlikely to play a regulatory role in phaseolin biosynthesis^{101,159}, in this system. A lack of correlation between activity and isoflavonoid biosynthesis has been described in cowpea hypocotyls responding to heavy metal ions or actinomycin-D¹⁷² producing kievitone (5) and in pea endocarp tissues treated with poly-L-arginine⁹³. Patridge and Keen¹⁵⁹ have reported similar results suggesting that PAL is either a simple wound/infection response and/or a non specific response to the fungus. Thus activation of PAL may not be correlated with the accumulation of 6a-hydroxyphaseolin (6) in flavonoid biosynthesis. However Yosikawa et al.¹⁷³ in their studies on biosynthesis and bio-



degradation of 6a-hydroxyphaseolin (6) by soybean hypocotyls infected with Phytophthora megasperma var sojae, have concluded that PAL may in fact be linked with glycoflin biosynthesis. Some other enzymes, apart from PAL, involved in the biosynthesis of isoflavonoids include hydroxycinnamate-CoA ligase, cinnamic acid-4-dioxygenase and O-methyltransferase (OMT)^{170,174,175}. The activity of these enzymes is shown to increase when flavonoid phytoalexins are induced^{170,172,175,176}.

Biosynthesis of medicarpin (2) involves conversion of phenylalanine to cinnamic acid which then gives chalcone after condensation and cyclization of malonate units. This step in flavone biosynthesis has been elegantly demonstrated by in vivo studies using purified chalcone synthetase which was previously misunderstood as flavone synthetase²⁴¹. Conversion

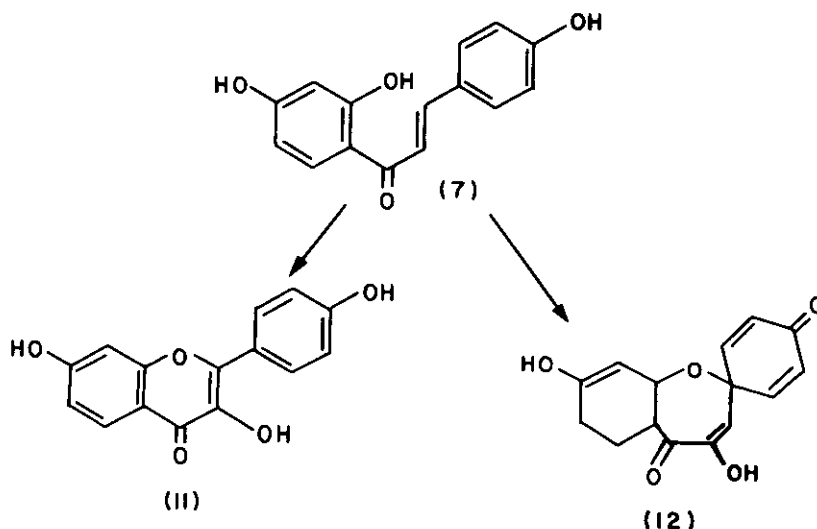
of chalcone to its corresponding flavone has been carried out *in vivo* by chalcone-flavone isomerase (CFI) thus establishing two independent stages in flavone biosynthesis. Isoflavone has been postulated as an isomerization product of chalcone by an aryl migration, which is then converted into pterocarpan by a series of reactions¹⁷⁷⁻¹⁷⁹. Previous reports^{174,159,180-182} about the unlinked role of chalcone-flavone isomerase and peroxidase in the biosynthesis of flavonoids need to be reassessed. A biosynthetic pathway leading to medicarpin (2) involving flavonoid biosynthetic enzymes in jackbean inoculated with *Pithomyces chartarum*¹⁷⁰ is outlined in scheme 2.



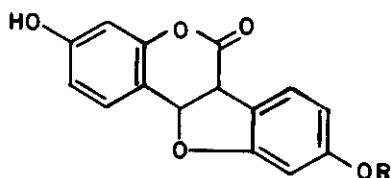
SCHEME 2

- | | |
|-----------------------------------|-------------------------------------|
| I. PAL | II. cinnamic acid-4-hydroxylase |
| III. p-coumaric CoA ligase | IV. chalcone synthetase |
| V. chalcone isomerase | VI. aryl migration |
| VII. daidzein-O-methyltransferase | VIII. genistein-O-methyltransferase |

Involvement of peroxidase in flavonoid biosynthesis has been demonstrated by purified soybean peroxidase and horseradish peroxidase which convert isoliquiritigenin (7) to 4'-7-dihydroxyflavon-3-ol (11) and a compound of structure^{159,-83-185} (12).



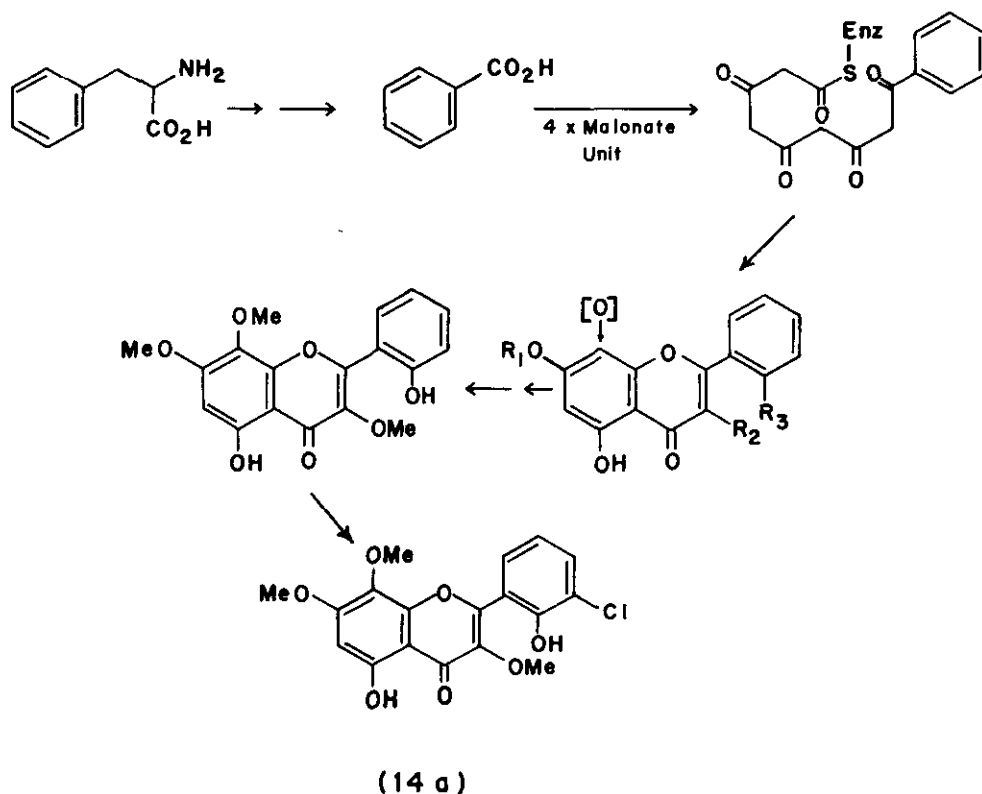
Slight structural variations may result in different controls in the biosynthesis of flavonoid/isoflavonoid phytoalexins. Thus Dixon and Bendall¹²⁹ have suggested a separate control for the synthesis of 5-hydroxy- and 5-deoxyflavonoid/isoflavonoid derivatives. These workers have suggested the presence of a flavone synthetase whose activity is regulated independently of the enzymes responsible for the formation of 5-deoxyisoflavan and coumestrol (13) which accumulate over longer time courses in *Phaseolus vulgaris* cell cultures treated with ribonuclease-A¹²⁹.



(13) : R = H

(14) : R = Me

Flavonoid secondary metabolites are ubiquitous in the plant kingdom and much known about the route by which they are synthesized. However Vinning and McInnes et al²⁴³ have recently reported a pathway of flavonoid biosynthesis differing from that of higher plants in that a C₆-C₁ precursor unit is condensed with four C₂ units. From tracer studies on the biosynthesis of chlorflavanin (14a) in *A. candidus*, these workers have proposed that the heterocyclic ring is formed before ring A is substituted at C-8 and while it is free to rotate at the enzyme surface. A proposed biosynthetic route of chlorflavanin according to Vinning and coworkers is produced in scheme 3.



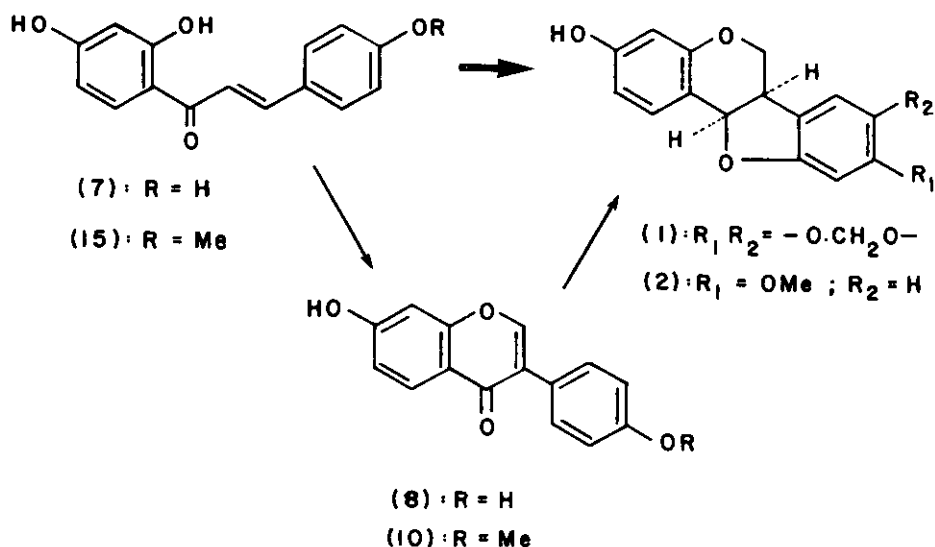
Scheme 3

Several views, for the migration of aryl ring in the isoflavonoid biosynthesis, have been presented. Dewick¹⁸⁶ concluded that methylation is an integral part of the aryl migration step, in the biosynthesis of these compounds. However Gustine et al.¹⁷⁰ have shown that isoliquiritigenin (7) and daidzein^{16,187} (8) are both methylated in presence of S-adenosyl-(¹⁴C-methyl)-methionine and an O-methyltransferase preparation. This has suggested that methylation could occur before or after the chalcone ring closure step. The occurrence of O-methyltransferase isozymes in soybean suspension cultures have been reported¹⁸⁸. One of these isozymes is specific for flavonoids and the other being specific for cinnamic acids. O-methyltransferase, specific for isoflavonoids, has been reported in chickpea¹⁸⁹.

Dewick and Martin¹⁹⁰ have proposed that 4'-hydroxyisoflavones, which are supposed to be derived from proton catalysed decomposition of the postulated spirodienone intermediate¹⁹¹, are not obligatory intermediates in the biosynthesis of 4'-methoxyisoflavones. These could arise by S-adenosylmethionine mediated decomposition of the spirodienone¹⁷⁷. However an enzyme catalysing 4'-methylation of daidzein (8) and genistein (9) has been reported¹⁸⁹. This is regarded as a minor route to the biosynthesis of formononetin¹⁹⁰.

Feeding experiments in CuCl₂ treated red clover seedlings have demonstrated that isoliquiritigenin (7) and formononetin (10) are readily incorporated into the pterocarpan phytoalexins 6aR, 11aR-demethylhomopterocarpin¹⁹² (2) and 6aR, 11aR-maackian (1). But 2,4-dihydroxy-4'-methoxychalcone (15) and daidzein (8) were poor precursors¹⁸⁶. The same four labelled compounds (7,8,10 & 15) have been examined as precursors of medicarpin (2), vestitol (22) and sativan (23) in Medicago sativa¹⁹⁰. It has been shown that isoliquiritigenin (7) and formononetin (10) but not 4'-methoxychalcone (15) and daidzein (8) are incorporated into these isoflavones including 9-O-methylcumestrol (14). However daidzein (8) and isoliquiritigenin (7) are incorporated into cumestrol (13) in Medicago sativa^{193,194} and Phaseolus vulgaris^{195,196}. Isoliquiritigenin (7) is also readily incorporated into formononetin^{186,193,197} (10), medicarpin¹⁸⁶ (2), maackian¹⁸⁶ (1) and rotenoid amorphenin¹⁷⁷. However daidzein (8) and methoxychalcone (15) are also poor precursors for maackian (1) and medicarpin (2). This is all in agreement to the conviction that methylation is an integral part of the aryl migration in isoflavonoid biosynthesis.

Medicarpin (2) and vestitol (22) are reported to be interconvertible in Medicago sativa and arise from a common precursor^{21,179} such as carbonium ion (18) or its mesomeric counter part (19) derived from isoflavanone^{179,198} (17). Feeding experiments suggested the existence of a common intermediate and simultaneous synthesis of medicarpin (2) and vestitol¹⁹⁹ (22). This has suggested a metabolic grid in Medicago sativa^{21,190,200}. Thus isoflav-3-ene type intermediate²⁰¹⁻²⁰⁴

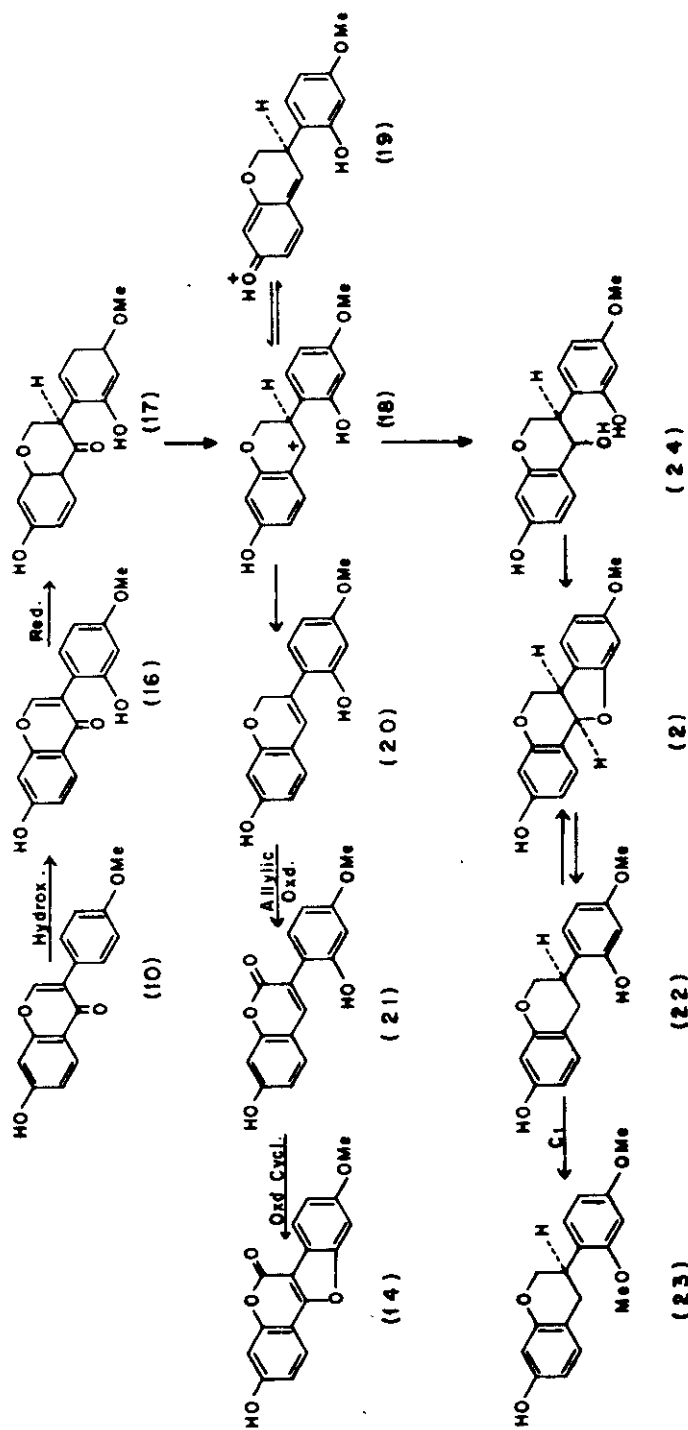


compounds are not important in the biosynthesis of *M. stiva* phytoalexins or as intermediates in the pterocarpin-2'-hydroxyisoflavan interconversion¹⁹⁹. On the other hand isoflav-3-ene type compounds are reported to play an important role in coumestans biosynthesis¹⁹⁹.

2',7-Dihydroxy-4'-methoxyisoflav-3-ene (20) has been considered as an intermediate in the biosynthesis of the phytoalexins medicarpin (2), (3R)-vestitol (22) and (3R)-sativan (23) in *Medicago sativa*¹⁷⁹. These compounds are derived by a stereospecific reduction sequence¹⁹² from 2',7-dihydroxy-4'-methoxyisoflavanone (17) via 2',7-dihydroxy-4'-methoxyisoflavone, (16) as reported earlier, in feeding experiments¹⁷⁹. Biosynthesis of these compounds is illustrated in scheme 4.

Isoflavans normally co-occur with the corresponding oxygenated pterocarpans^{94,137,205,206} as shown in scheme 4. Pterocarpans may be produced by an oxidative process involving 2'-hydroxyisoflavans²⁰⁷. Feeding experiments in red clover (*Trifolium pratense*)^{186,207} have suggested that the biosynthetic pathway to medicarpin (2) proceeds via the isoflavone formononetin (10) followed by 2'-hydroxylation to isoflavone (16) and finally reduction to isoflavanone (17). This isoflavanone undergoes presumable reduction to isoflavanol (24) which subsequently cyclizes to medicarpin¹⁷⁹.

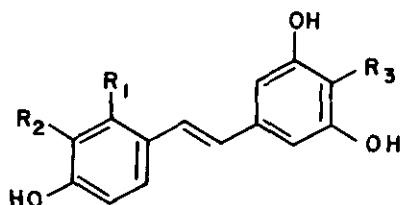
Reductive ring opening of a pterocarpin to a 2'-hydroxyisoflavan²⁰⁸⁻²¹¹, and methylation of an isoflavonoid are among the demonstrated metabolic processes initiated by fungi^{116,212}. Biochemically, the pterocarpin \longrightarrow 2'-hydroxyisoflavan conversion has been demonstrated during fungal detoxication of pterocarpin phytoalexins, such as maackiain (1), medicarpin (2) and phaseollin²⁰⁹⁻²¹¹ (4). Red clover is reported to synthesize only pterocarpin phytoalexins producing



Scheme 4

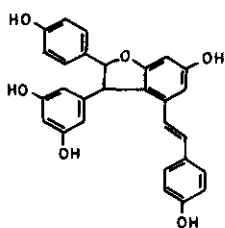
maackiain (1) and medicarpin²¹³. However the isoflavans in this plant are speculated to be induced since the plant has the ability to convert vestitol (22) to medicarpin (2). It is note worthy that isoflavan phytoalexins with 4',5'-methylenedioxy substituent have not been found along with their corresponding pterocarpan²¹⁴. Exception to this has been reported by Dewick¹⁷⁹ who has described three pterocarpan precursors namely formononetin (10), liquiritigenin, and isoliquiritigenin (7) accompanying maackian (1) and medicarpin (2) in Trigonella species.

Trans-stilbene and bisarylpropanoid phytoalexins are closely related to isoflavan phytoalexins. At least six trans-stilbene phytoalexins from plants both fungally infected and incuded with abiotic treatment have been isolated and characterized. These compounds are all trans-resveratrol analogues (25-30), accumulated in Grapvine^{215,216}, Arachis hypogaea²¹⁷⁻²¹⁹ and Morus alba^{217,220} Linne.

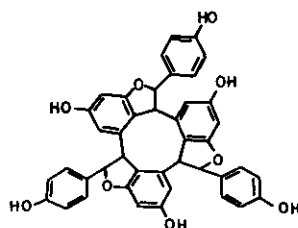


- 25: Resveratrol: $R_1=R_2=R_3=H$
 26: 4'-Isopentenylresveratrol: $R_1=R_2=H$; $R_3=$ isopentenyl
 27: 2-Hydroxyresveratrol: $R_2=R_3=H$; $R_1=OH$
 28: 4'-Isopentenyl-2-hydroxyresveratrol: $R_1=OH$; $R_2=H$; $R_3=$ isopentenyl
 29: 4'-Isopentenyl-3-hydroxyresveratrol: $R_1=H$; $R_2=OH$; $R_3=$ isopentenyl
 30: 4'-(3-methyl-but-1-enyl)-resveratrol: $R_1=R_2=H$; $R_3=$ 3-methyl-but-1-enyl

Resveratrol has been predicted²¹⁵ as a biosynthetic precursor of the viniferins ξ (31) and α (32), antifungal compounds characteristic of the family Vitaceae.

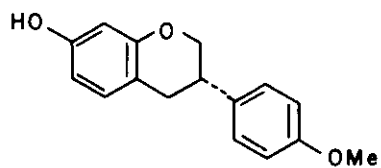


(31)

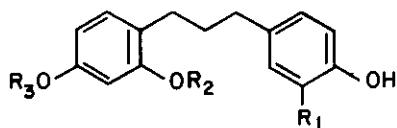


(32)

The co-occurrence of isoflavan broussin (33) and bisarylpropanoid broussin-C (34) in mulberry (*Broussonetia papyrifera* vent.) has suggested²²¹ a close biosynthetic relationship between two types of compounds. Broussin-A (35) and broussanin-B (36) have been isolated from mulberry in response to *Fusarium solani* f. sp. *mori*^{221,222}. Mulberry contains antifungal compounds albanins F (37) and G²²³ (38), considered to be formed by Diels-Alder type of reactions *in vivo*. Chalcomoracin (39) is also considered to be formed by a Diels-Alder type of enzymatic reaction process or morachalcone-A (40) and dehydromoracin-C (41) or its equivalents. The co-occurrence of morachalcone-A (40), moracin-D, equivalent to moracin-C (41), and chalcomaracin (39) as minor phytoalexins in the infected cortical tissue of mulberry shoots has supported this hypothesis²²⁴.



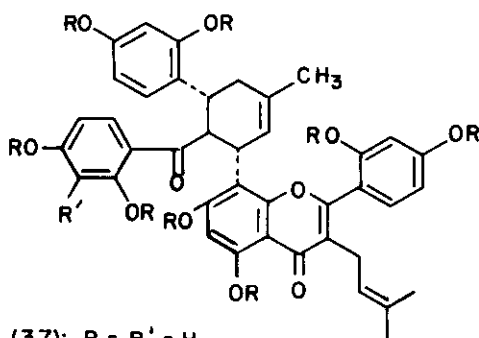
(33)



(34): $R_2 = R_3 = H$; $R_1 = \text{isopentenyl}$

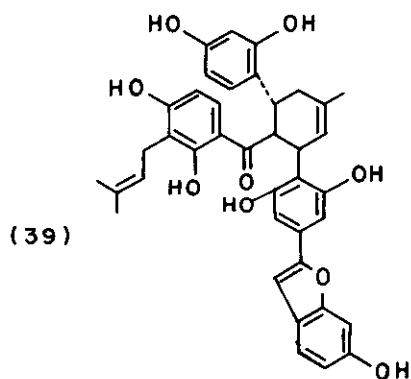
(35): $R_1 = R_2 = H$; $R_3 = \text{Me}$

(36): $R_1 = R_3 = H$; $R_2 = \text{Me}$

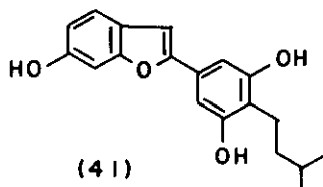


(37): $R = R' = H$

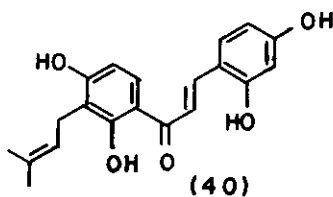
(38): $R = H$, $R' = \text{prenyl}$



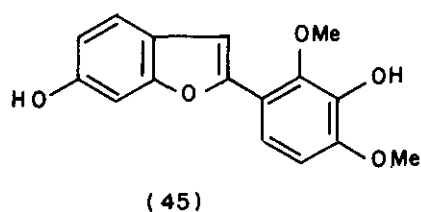
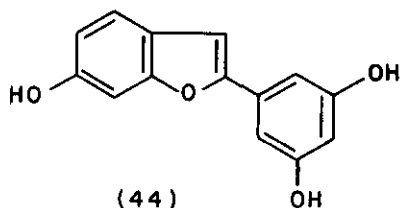
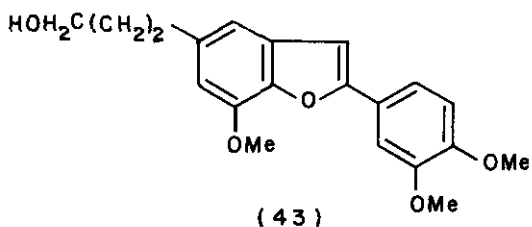
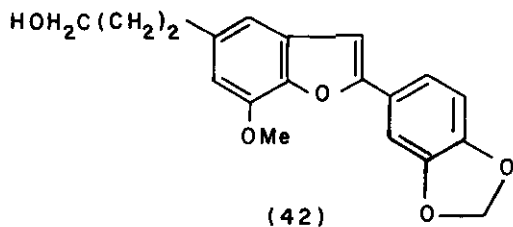
(39)



(41)

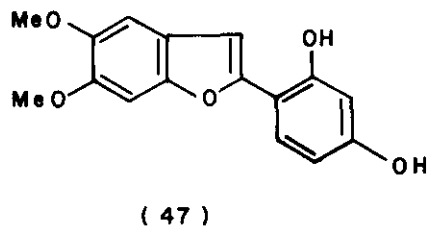
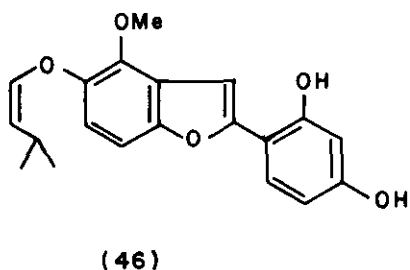


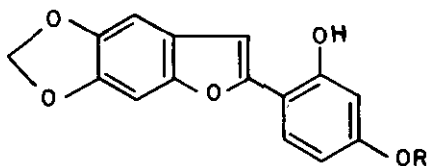
(40)



Structural diversity of arylbenzofurans has suggested that these compounds may arise by a number of different routes. Most commonly accepted route involves oxidative cyclization process of hydroxystilbenes isolated from the same or related source. Thus co-occurrence of 2-(3,5-dihydroxyphenyl)-6-hydroxybenzofuran (44) with resveratrol (27) in *Morus laevigata*²³¹ and the phytoalexins moracins A-H in *Morus alba* in response to *Fusarium solani* f. sp. *mori*²²⁵⁻²²⁷ has given substantial support to this pathway. A close biogenetic relationship between the co-occurring isoflavan (33) and bisarylpropanoid (34), has suggested that bisarylpropanoids, which may arise after reduction of chalcone derivatives, are also involved in the biosynthesis of arylbenzofurans²⁴². Thus Martin and Dewick have suggested²⁴² that egonol (42) and homoegonol (43), from *styrax* species²²⁸⁻²³⁰ are formed from bisarylpropanoids by loss of a carbon atom.

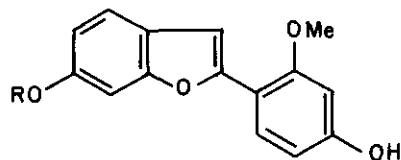
However if a cyclization process precedes a reduction of chalcone, a flavone may result





(48) : R = H

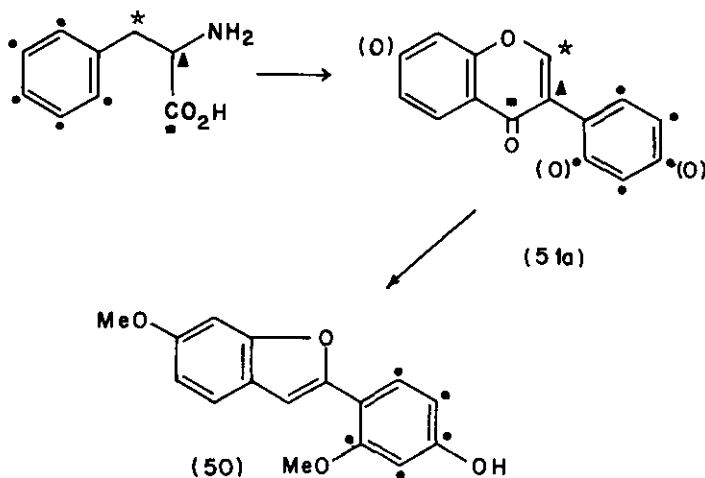
(49) : R = Me



(50) : R = Me

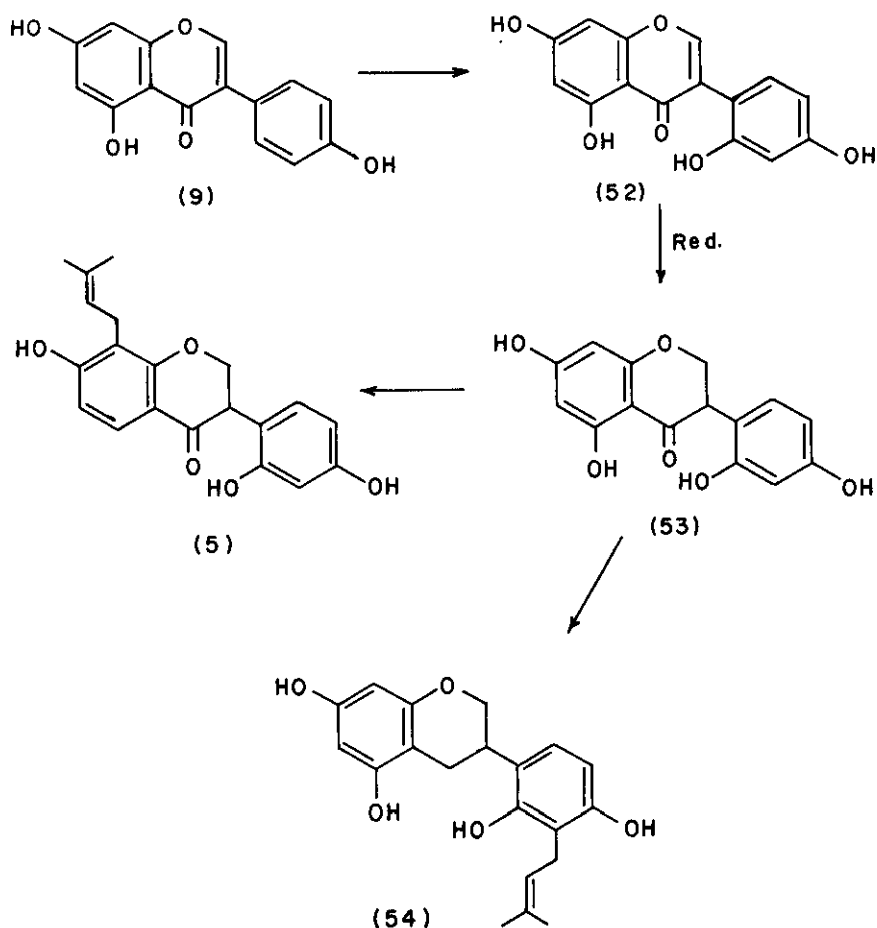
(51) : R = H

which could then lose a carbon atom to give arylbenzofurans. This pathway has been supported by labelling studies for the biosynthesis of vignafuran (50) from leaves of cowpea, *Vigna unguiculata* infected with *Colletotricum lindemuthianum*^{235,242} and *Lablab niger* infected with *Helminthosporium carbonum*²³⁶. Thus incorporation of labelled phenylalanine into vignafuran (50) has suggested the loss of a carbon atom C-3 of phenylalanine and that vignafuran (50) is derived from an isoflavonoid precursor (51a). Loss of a carbon atom from coumestan, during biosynthesis of vignafuran has also been reported^{234,239}. Pterofuran (45) from *Pterocarpus indicus*²³², neoraufurane (46) from *Neorautanenina edulis*²³³, 2-(2,4-dihydroxyphenyl)-5,6-dimethoxybenzofuran (47) from *Myroxylon balsamum*²³⁴ and 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxybenzofuran (48) and its methyl ether (49), from *Sophora tomentosa*²³⁵ are all speculated to be derived from corresponding flavonoid precursors by loss of carbon atom²⁴².



2'-Hydroxylation and isoprenylation are a common process in isoflavonoid phytoalexin biosynthesis. Thus phaseolutone (54), a metabolite of french bean (*Phaseolus vulgaris* L) in response to *Monilinia fructicola* (Wint.) Honey, is proposed²⁴⁰ to be synthesized from its co-occurring isoflavones such as genistein (9) and 2'-hydroxygenistein (52). A direct synthesis of kievitone (5) in *Phaseolus vulgaris* L. is also suggested²⁴⁰ through hydroxylation and prenylation of its co-occurring isoflavones.

Although a number of research groups are actively involved in the study of phytoalexins and their role in plant defense mechanisms, more comprehensive *in vivo* studies are warranted to understand their biosynthetic formation. This will bring to light the role played by these compounds in plant protection.



References

1. I.A.M. Cruickshank, Ann. Rev. Phytopathol., 1963, 1, 351.
2. J.L. Ingham, Bot. Rev., 1972, 38, 343.
3. J. Kuc, Ann. Rev. Phytopathol., 1972, 10, 207.
4. K. Tomiyama, T. Sakuma, N. Ishizaka, N. Sato, N. Katsui, M. Takasugi and T. Masamune, Phytopathology, 1968, 58, 115.
5. J.A. Bailey, Physiol. Plant Pathol., 1974, 4, 477.
6. J.A. Bailey and B.J. Deverall, Physiol. Plant Pathol., 1971, 1, 435.
7. J.L. Varns, J. Kuc and E. Williams, Phytopathology, 1971, 61, 174.
8. L.J. Duczek and V.J. Higgins, Canad. J. Bot., 1976, 54, 2609.
9. M. Yoshikawa, K. Yamahachi and H. Masago, Physiol. Plant Pathol., 1978, 12, 73.
10. E.W.B. Ward, C.H. Unwin and A. Stoessl, Canad. J. Bot., 1973, 51, 2327.
11. N. Sato and K. Tomiyama, Ann. Phytopathol. Soc. Japan., 1969, 35, 202.
12. J.A. Bailey and J.L. Ingham, Physiol. Plant Pathol., 1971, 1, 451.
13. A.E. Arinze and I.M. Smith, Physiol. Plant Pathol., 1980, 17, 145.
14. W.L. Klarman and F. Hammerschlag, Phytopathology, 1972, 62, 719.
15. P. Stholasuta, J.A. Bailey, V. Severin and B.J. Deverall, Plant Physiol., 1971, 1, 177.
16. N.T. Keen and B.W. Kennedy, Physiol. Plant Pathol., 1974, 4, 173.
17. J.E. Cross, B.W. Kennedy, J.W. Lambert and R.L. Copper, Plant Disease Reports, 1966, 50, 557.
18. C. Leben, G.C. Daft and A.F. Schmitthenner, Phytopathology, 1968, 58, 1143.
19. A.R. Ayer, B. Valent, J. Ebel and P. Albersheim, Plant Physiol., 1976, 57, 766.
20. I.A.M. Cruickshank and D.R. Perrin, Life Sciences, 1968, 1, 449.
21. A. Van. Dijkman and A. Kaars-Sijpesteijn, Physiol. Plant Pathol., 1973, 3, 57.
22. J.A. Frank and J.D. Paxton, Phytopathology, 1971, 61, 954.
23. N.T. Keen, Science, 1975, 187, 74.
24. N.T. Keen, J. Partridge and A. Zaki, Phytopathology, 1972, 62, 768.
25. N.T. Keen, M.C. Wang, S.B. Garcia, G.A. Zentmyer, Physiol. Plant Pathol., 1975, 7, 91.
26. P.C. Mercer, R.K.S. Wood and A.D. Greenwood, Physiol. Plant Pathol., 1974, 4, 291.
27. R.A. Skipp and B.J. Deverall, Physiol. Plant Pathol., 1973, 3, 299.
28. J.L. Varns, W.W. Currier and J. Kuc, Phytopathology, 1971, 61, 968.
29. P. Albersheim and B.S. Valent, J. Cell. Biol., 1978, 78, 627.
30. A.A. Bell, Phytopathology, 1967, 57, 759.

31. J.B. Harborne, T.J. Mabry and H. Mabry, "The Flavonoids", Chapman and Hall, London, 1975.
32. R.S. Burden, and J.A. Bailey, Phytochemistry, 1975, 14, 1389.
33. R.L. Lyne, L.J. Mulheirn and D.P. Leworthy, J. Chem. Soc. Chem. Comm., 1976, 497.
34. R.L. Lyne and L.J. Mulheirn, Tetrahedron Letters, 1978, 3127.
35. A.R. Ayers, J. Ebel, F. Finelli, N. Berger and P. Albersheim, Plant Physiol., 1976, 57, 751.
36. P. Moesta and H. Grisebach, Nature, 1980, 286, 710.
37. J.A. Hargreaves, Plant Pathol., 1979, 15, 279.
38. I.A.M. Cruickshank and D.R. Perrin, Life Sciences, 1963, 3, 680.
39. D.R. Perrin, Tetrahedron Letters, 1964, 1, 29.
40. W.L. Klarman and J.B. Sanford, Life Science, 1968, 7, 1095.
41. V.J. Higgins and R.L. Millar, Phytopathology, 1968, 58, 1377.
42. F.J. Muehlbauer and C. Sander, Pisum. Newsletter, 1975, 7, 32.
43. R.A. Nilan, E.G. Sideris, A. Klenoffs, C. Sander and C.F. Konzak, Mutation Research, 1973, 17, 42.
44. W.Q. Loegering, Hereditas Suppl., 1966, 11, 167.
45. T. Minamikawa and I. Uritani, Arch. Biochem. Biophys., 1964, 108, 573.
46. K.O. Muller, Aust. J. Sci., 1958, 11, 275.
47. G. Loebenstein, S. Rabina and T. Van-Praag, Virology, 1968, 34, 264.
48. S. Von Broemsen and L.A. Hadwiger, Phytopathology, 1969, 59, 1055.
49. A.A. Bell and M.N. Christensen, Phytopathology, 1968, 58, 883.
50. R. Adams, T.A. Geissman and J.A. Edwards, Chem. Rev., 1960, 60, 555.
51. C.H. Boatner, "Cottonseed", Ed. A.E. Bailey, Inter. Science Publishers, N.Y., 1948, p. 213.
52. A.A. Bell, Phytopathology, 1969, 59, 1119.
53. I.A.M. Cruickshank, Pontificiae Academiae Scientarum Scripta Varia, 1977, No. 41.
54. L.A. Hadwiger and S. Fulger, Phytopathology, 1967, 57, 1005.
55. N.T. Keen and B. Bruegger, Amer. Chem. Soc. Symp. Series, 1977, 62, 26.
56. A.J. Anderson-Prouty and P. Albersheim, Plant Physiol., 1975, 56, 286.
57. D.C. Hildebrand and M.M. Schroth, Phytopathology, 1968, 58, 354.
58. A.R. Ayers, J. Ebel, B. Valent and P. Albersheim, Plant Physiol., 1976, 57, 760.
59. N.T. Keen, Science, 1975, 187, 74.
60. K. Cline, M. Wade and P. Albersheim, Plant Physiol., 1978, 62, 918.
61. P. Stoessl and R. Hohl, Mycopathologia, 1981, 73, 153.
62. G.D. Lyon, Physiol. Plant pathol., 1972, 2, 411.
63. J. Kuc, "Specificity in Plant Diseases", Ed. R.K.S. Wood and A. Graniti, Plenum Publishing Corp. N.Y., 1976, p. 253.

64. A.J. Anderson, Phytopathology, 1978, 68, 189.
65. D.L. Daniels and I.A. Hadwiger, Physiol. Plant Pathol., 1976, 8, 9.
66. N. Lisker and J. Kuc, Phytopathology, 1977, 67, 1356.
67. A. Anderson, Canad. J. Bot., 1973, 56, 2247.
68. A. Anderson, Canad. J. Bot., 1980, 58, 2343.
69. Bartnicki-Garcia, Ann. Rev. Microbiol., 1968, 22, 87.
70. E.J. Nichols, J.M. Beckman and L.A. Hadwiger, Plant Physiol., 1980, 66, 199.
71. L.A. Hadwiger, Plant Physiol., 1969, 63, 5.
72. R. Michell, Phytopathology, 1963, 53, 1068.
73. G.R. Dixon and G.F. Pegg, Trans. Br. Mycol. Soc., 1969, 53, 109.
74. G.F. Pegg, 2nd Int. Cong: Plant Pathol. Minnea Polis. Abst., 0968, 1973.
75. G.F. Pegg and J.C. Vessey, Physiol. Plant Pathol., 1973, 3, 207.
76. W. Grassmann, R. Zechmeister, R. Bender and G. Toth, Berichte, 1934, 67, 1.
77. R.F. Powning and A. Irzykiewicz, Comp. Biochem. Physiol., 1965, 14, 127.
78. K.M.L. Agarwal and O.M. Bahl, J. Biol. Chem., 1968, 234, 103.
79. J.J. Skujins, Arch. Biochem. Biophys., 1965, 111, 358.
80. J.B. Howard and A.N. Glazer, J. Biol. Chem., 1967, 242, 5715.
81. H. Mandels, F.W. Marrish and E.T. Reese, Phytochemistry, 1967, 6, 1097.
82. M. Yashikawa, M. Matawa and H. Masago, Plant Physiol., 1981, 67, 1032.
83. A.E. Clarke and B.A. Stone, Phytochemistry, 1962, 1, 175.
84. L.I. Chalova, O.L. Ozeretskovskaya, L.A. Yurganova, V.G. Baramidze, M.A. Protsenko, Y.U.T. D'Yakov and L.V. Metlitskii, Dokl. Akad. Nauk. SSSR., 1976, 230, 722.
85. L.V. Melitskii, O.L. Ozeretskovskaya, L.A. Yurganova, O.N.S. Eva, L.I. Chalova and Y.T.D. Yakov, Dokl. Acad. Nauk. SSSR., 1976, 226, 1217.
86. N.T. Keen, E.T. Long, B. Bruegger and M. Holliday, Physiol. Plant Pathol., 1981, 18, 325.
87. A.A. Bell and J.T. Presley, Phytopathology, 1969, 59, 1147.
88. R.A. Dixon and K.W. Fuller, Physiol. Plant Pathol., 1977, 11, 287.
89. M. Stekoll and C.A. West, Plant Physiol., 1978, 61, 38.
90. A.I. Zaki, N.T. Keen and D.C. Erwin, Phytopathology, 1972, 62, 1402.
91. M. Wade and P. Albersheim, Proc. Natl. Acad. Sci. U.S.A., 1979, 76, 4433.
92. I.A.M. Cruickshank and D.R. Perrin, Life Science, 1969, 7, 449.
93. L.A. Hadwiger and M.E. Schwochau, Biochem. Biophys. Research Comm., 1970, 38, 683.
94. L.A. Hadwiger, A. Jafri, Von. Broembson and R. Eddy, Plant Physiol., 1974, 53, 52.
95. P.J.G.M. Dewit and P.H.M. Roseboom, Physiol. Plant Pathol., 1980, 16, 391.

96. J.M. Dow and J.A. Callow, Physiol. Plant Pathol., 1979, 15, 27.
97. N.T. Keen and M. Legrand, Physiol. Plant Pathol., 1980, 17, 175.
98. N.T. Keen, "Biochem. and Cytology of Plant-Parasite Interaction", Ed. K. Tomiyama, 1976, p. 84.
99. A.J. Anderson, Phytopathology, 1979, 69, 913.
100. N.T. Keen and M. Long, Physiol. Plant Pathol., 1972, 2, 307.
101. N.T. Keen, Physiol. Plant Pathol., 1971, 1, 265.
102. C. Ballou, Advances in Microbial. Physiol., 1976, 14, 93.
103. O. Bedwin, Proc. Royal Soc. London, 1979, 205, 271.
104. R.G. Brown and W.C. Kimmins, Inter. Rev. Biochem., 1977, 13, 183.
105. E.A. Clark, P. Gleeson, S. Harrison and R.B. Knox, Proc. National Acad. Sci. U.S.A., 1979, 76, 3358.
106. B.A. Cunningham, Scientific American, 1977, 237, 96.
107. K. Muller and G. Gerisch, Nature, 1978, 274, 445.
108. D.R. Perrin and I.A.M. Cruickshank, Aust. J. Biol. Sci., 1965, 18, 803.
109. J.A. Bailey, Annals of Applied Biology, 1969, 64, 315.
110. E. Chalutz and M.A. Stahman, Phytopathology, 1969, 59, 1972.
111. J.A. Bailey, Phytochemistry, 1969, 8, 1393.
112. L.A. Hadwiger and M.E. Schwochau, Plant Physiol., 1971, 47, 346.
113. S.L. Von Broembsen and L.A. Hadwiger, Physiol. Plant Pathol., 1972, 2, 207.
114. M.E. Schwochal and L.A. Hadwiger, Arch. Biochem. Biophys., 1969, 134, 34.
115. L.A. Hadwiger and M.E. Schwochau, Plant Physiol., 1971, 47, 588.
116. J.L. Ingham, Phytochemistry, 1976, 15, 1489.
117. J.L. Ingham, Canad. J. Bot., 1978, 56, 2230.
118. K.O. Muller, Phytopathology, Z., 1956, 27, 237.
119. P. Langcake, D. Cartwright, D.P. Leworthy, R.J. Pryce and J.P. Ride, Neth. J. Plant. Pathol. (suppl.), 1978, 83, 153.
120. J.E. Rahe and R.M. Arnold, Canad. J. Bot., 1975, 53, 921.
121. J.A. Hargreaves and J.A. Bailey, Physiol. Plant Pathol., 1978, 13, 89.
122. J.W.D.M. Henfling, N. Lisker and J. Kuc, Phytopathology, 1978, 68, 857.
123. M. Reuveni and Y. Cohen, Physiol. Plant Pathol., 1978, 12, 179.
124. S.F. Yang, H.K. Pratt, "Biochemistry of Wounded Plant Tissues", Ed. G. Kahl, Water de Gruyter, N.Y., 1978.
125. F. Koch, M. Baur, M. Burba and E.F. Elstner, Phytopathology, 1980, 98, 40.

126. P. Montalbini and E.F. Elstner, Planta, 1977, 135, 301.
127. B.C. Carton, C.E. Peterson and N.E. Tolbert, Plant Physiol., 1961, 36, 550.
128. I. Uritani, M. Uritani and H. Yamada, Phytopathology, 1960, 50, 30.
129. R.A. Dixon and D.S. Bendall, Physiol. Plant Pathol., 1978, 13, 283.
130. J.A. Bailey and M. Berthier, Phytochemistry, 1981, 20, 188.
131. D.W. Cartwright, P. Langcake, R.J. Pryce, D.P. Leworthy, and J.P. Ride, Phytochemistry, 1981, 20, 535.
132. D.W. Cartwright and P. Langcake, Physiol. Plant Pathol., 1980, 17, 259.
133. A.A. Bell and J.T. Presley, Phytopathology, 1969, 59, 1141.
134. A. Fraile, F.G. Arenal and E.M. Sagasta, Physiol. Plant Pathol., 1980, 16, 9.
135. M. Yoshikawa, Nature, 1978, 275, 546.
136. J.L. Ingham and J.B. Harborne, Nature, 1976, 260, 241.
137. J.B. Harborne and J.L. Ingham "Biochemical Aspects of Plant and Animal Coevolution", Ed. J.B. Harborne, Academic press, N.Y., 1978, p. 343.
138. J. Van Den Heuvel, Neth. J. Plant Pathology, 1976, 82, 153.
139. J.L. Ingham, Biochem. Syst. Ecol., 1979, 7, 29.
140. P.J.G.M. Dewit and W. Flach, Physiol. Plant Pathol., 1979, 15, 257.
141. R.N. Letcher, D.A. Widdowson, B.J. Deverall and J. W. Mansfield, Phytochemistry, 1970, 9, 249.
142. J.P. Goodlife and J.B. Heale, Physiol. Plant Pathol., 1978, 12, 27.
143. V.K. Harding and J.B. Heale, Physiol. Plant Pathol., 1980, 17, 227.
144. F. Bohlman "The Biology and Chemistry of the Umbelliferae", Ed. V.H. Heywood, Supplement to Botanical Journal of the Linnean Society, 1971, 64, 279.
145. V. Harding and H.B. Heale, Annals of Applied Biol., 1978, 89, 348.
146. J.B. Heale, V. Harding, K. Dodd and P.B. Gaban, Annals of Applied Biol., 1978, 89, 310.
147. T. Boller, R.C. Herner and H. Kende, Planta, 1979, 145, 293.
148. L.A. Hadwiger, S.L. Hess and S.V. Broemben, Phytopathology, 1970, 60, 332.
149. D.H. Daniels and L.A. Hadwiger, Physiol. Plant Pathol., 1976, 8, 9.
150. L.A. Hadwiger, Phytochemistry, 1966, 5, 523.
151. P.J. Kuhan and D.A. Smith, Annals Applied Biol., 1975, 362.
152. S.L. Hess and M. Schwochau, Phytopathology, 1969, 59, 1030.
153. R.L. Millar and V.J. Higgins, Phytopathology, 1968, 58, 1377.
154. L.A. Hadwiger, Biochem. Biophys. Res. Comm., 1972, 46, 71.
155. L.A. Hadwiger, Neth. J. Plant. Pathol., 1968, 74, 163.
156. E.J. Rahe, J. Kuc, C.M. Chuang and E.B. Williams, Neth. J. Plant Pathol., 1969, 75, 58.

157. M. Zucker, Plant Physiol., 1965, 40, 779.
158. T. Minamikawa and I. Uritani, Agr. Biol. Chem. (Tokyo), 1965, 29, 1021.
159. J.E. Partridge and N.T. Keen, Phytopathology, 1977, 67, 50.
160. N. Amrhein and M.H. Zenk, Naturwissenschaften, 1970, 57, 312.
161. D.C. Walton, Plant Physiol., 1968, 43, 1120.
162. D.C. Walton, Plant Physiol., 1968, 43, 467.
163. J. Ebel, A.R. Ayers and P. Albersheim, Plant Physiol., 1976, 57, 775.
164. R.A. Dixon and K.W. Fuller, Physiol. Plant Pathol., 1976, 9, 299.
165. L.L. Creasy and M. Zucker, Recent Advances in Phytochem., 1974, 8, 1.
166. D.R. Perrin and W. Bottomley, J. Amer. Chem. Soc., 1962, 84, 919.
167. A. Stoessl, Canad. J. Biochem., 1972, 50, 107.
168. S.G. Pueppke and H.D. Van Ethen, J. Chem. Soc. Perkin Trans. 1., 1975, 946.
169. R.E. Carlson and D.H. Dolphin, Phytochemistry, 1981, 20, 2281.
170. D.L. Gustine, R.T. Sherwood and C.P. Vance, Plant Physiol., 1978, 61, 226.
171. R.A. Dixon and K.W. Fuller, Physiol. Plant Pathol., 1978, 12, 279.
172. C.B. Munn and R.B. Drysdale, Phytochemistry, 1975, 14, 1303.
173. M. Yashikawa, K. Yamauchi and M. Massago, Physiol. Plant Pathol., 1979, 14, 157.
174. W.G. Rathmell, Physiol. Plant Pathol., 1973, 3, 259.
175. K. Hahlbrock, H. Grisebach "The Flavonoids" part 2, Ed. J.B. Harborne, J.J. Mabry and H. Mabry, Academic Press, New York, 1975, p. 866.
176. N.T. Keen, Phytopathology, 1972, 62, 1365.
177. L. Combie, P.H. Dewick and D.A. Whiting, J. Chem. Soc. Perkin Trans., 1973, 1285.
178. P.M. Dewick, Phytochemistry, 1977, 16, 93.
179. P.M. Dewick and M. Mortin, J. Chem. Soc. Chem. Comm., 1976, 16, 1937.
180. K. Hahlbrock, E. Wong, Lischill and H. Grisebach, Phytochemistry, 1970, 9, 949.
181. C.R. Curtis and N.M. Barnett, Canad. J. Bot., 1974, 46, 2037.
182. W.G. Rathmell and D.S. Bendall, Biochem. J., 1972, 127, 125.
183. E. Wong and T.M. Wilson, Hoppe Seyler's Z. Physiol. Chem., 1972, 353, 132.
184. E. Wong and T.M. Wilson, Phytochemistry, 1976, 15, 1325.
185. E. Wong and T.M. Wilson, Phytochemistry, 1972, 11, 875.
186. P.M. Dewick, Phytochemistry, 1975, 14, 979.
187. N.T. Keen, Phytopathology, 1978, 68, 1237.
188. J. Poulton, J. Grisebach, J. Ebel, B. Schaller - Hekelel and Hahlbrock, Arch. Biochem. Biophys., 1976, 173, 301.

189. H. Wengenmayer, J. Ebel and H. Grisebach, Eur. J. Biochem., 1974, 50, 135.
190. P.M. Dewick and M. Martin, Phytochemistry, 1979, 18, 591, 597.
191. A. Pelter, J. Bradshaw and R.E. Warren, Phytochemistry, 1971, 10, 835.
192. P.M. Dewick and D. Ward, J. Chem. Soc. Chem. Comm., 1977, 338.
193. H. Grisebach and W. Barz, Naturforsch. Z., 1964, 19B, 569.
194. W. Barz and H. Grisebach, Naturforsch. Z. Teil., 1966, 21B, 1113.
195. P.M. Dewick, W. Barz and H. Grisebach, Phytochemistry, 1970, 9, 775.
196. J. Berlin, P.M. Dewick, W. Barz and H. Grisebach, Phytochemistry, 1972, 11, 1689.
197. H. Grisebach and L. Patschke, Chem. Ber., 1960, 93, 2326.
198. P.M. Dewick, J. Chem. Soc. Chem. Comm., 1975, 656.
199. M. Martin and P.M. Dewick, Tetrahedron Letters, 1978, 2341.
200. J.D. Bu'Lock, "The Biosynthesis of Natural Products", McGraw Hill, London, 1965, p. 82.
201. A.J. Brink, G.J.H. Rall and J.P. Engelbrecht, Tetrahedron, 1974, 30, 311.
202. L. Jurd, Tetrahedron Letters, 1976, 1741.
203. L. Jurd, Tetrahedron Letters, 1977, 723.
204. D.M.X. Donnelly and P.D. Kavanagh, Phytochemistry, 1974, 13, 2587.
205. H.D. Van Etten and S.G. Pueppke, Isoflavonoid phytoalexin, "Biochemical Aspects of Plant-Parasite Relationships", Ed. J. Friend and D.R. Threfall, Acad. press, London, 1976, p. 239.
206. J.L. Ingham, Biochem. Syst. Ecol., 1978, 6, 217.
207. M. Cornia and L. Merlini, J. Chem. Soc. Chem. Comm., 1975, 428.
208. E. Wong, "The Flavonoid", Ed. J.B. Harborne, T.J. Mabry and H. Mabry, Chapman and Hall, London, 1975, p. 772.
209. P.W. Steine and R.L. Millar, Phytopathology, 1974, 64, 586.
210. Z. Kiraly, B. Barna and T. Ersek, Nature, 1972, 239, 456.
211. V.J. Higgins, H. Stoessl and M.C. Heath, Phytopathology, 1974, 64, 105.
212. J.N. Bilton, J.R. Debnam and I.M. Smith, Phytochemistry, 1976, 15, 1411.
213. P.M. Dewick, Phytochemistry, 1978, 17, 1751.
214. J.L. Ingham and P.M. Dewick, Phytochemistry, 1980, 19, 1767.
215. P. Langcake and R.J. Pryce, Experientia, 1977, 33, 151.
216. P. Langcake and R.J. Pryce, Physiol. Plant Pathol., 1976, 9, 77.
217. J.L. Ingham, Phytochemistry, 1976, 15, 1791.
218. N.T. Keen and J.L. Ingham, Phytochemistry, 1976, 15, 1794.
219. G.E. Aguamah, P. Langcake, D.P. Leworthy, J.A. Page, R.J. Pryce and R.N. Strange,

Phytochemistry, 1981, 20, 1381.

220. V.H. Deshpande, R. Srinivasan and A.V. Rama Rao, Indian J. Chem., 1975, 13, 453.
221. M. Takasugi, Y. Kumagai, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1980, 1459.
222. M. Takasugi, M. Anetai, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1980, 339
223. M. Takasugi, S.I. Ishikawa, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1980, 1577.
224. M. Takasugi, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1980, 1573.
225. M. Takasugi, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, Tetrahedron Letters, 1979, 4675.
226. M. Takasugi, S. Nagao, S. Ueno, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1978, 1239.
227. M. Takasugi, S. Nagao and T. Masamune, Tetrahedron Letters, 1978, 797.
228. R. Segal, I. Milo-Goldzweig, S. Sokoloff and D.V. Zaitschek, J. Chem. Soc., 1967, 2402.
229. R.S. McCredie, E. Ritchie and W.C. Taylor, Aust. J. Chem., 1969, 22, 1011.
230. O.R. Gottlieb, Phytochemistry, 1972, 11, 1537.
231. K. Venkataraman, Phytochemistry, 1972, 11, 1571.
232. R.G. Cooke and I.D. Rae, Aust. J. Chem., 1964, 17, 379.
233. A.J. Brink, G.J.H. Rall and J.P. Engelbrecht, Tetrahedron, 1974, 30, 311.
234. A.B. De Oliverira, M. Iracema, L.M. Hadruga and O.R. Gottlieb, Phytochemistry, 1978, 17, 593.
235. M. Komatsu, I. Yokoe and Y. Shirataki, Chem. Pharm. Bull., 1978, 26, 1274.
236. J.L. Ingham, Z. Naturforsch. Teilc., 1977, 32, 1018.
237. J.L. Ingham and P.M. Dewick, Phytochemistry, 1978, 17, 535.
238. J.L. Ingham, Phytochemistry, 1977 16, 1279.
239. T.R. Sheshadri, Phytochemistry, 1972, 11, 881.
240. M.D. Woodward, Phytochemistry, 1979, 18, 363.
241. W. Heller and K. Hahlbrock, Arch. Biochem. Biophys., 1980, 200, 617.
242. M. Martin and P.M. Dewick, Phytochemistry, 1979, 18, 1309
243. M.K. Burns, J.M. Coffin, I. Kurobane, L.C. Vinning, A. Gavin McClines, D.G. Smith and J.A. Walter, J. Chem. Soc., Perkin I, 1981, 1411

Acknowledgement: The authors gratefully acknowledge a financial grant No. SB010 from the Research Management Unit, Kuwait University to accomplish this work.

Received, 17th February, 1982