BIOSYNTHESIS OF ISOFLAVONOID AND RELATED PHYTOALEXINS

Muhammad Afzal* and Galib Al-Oriquat

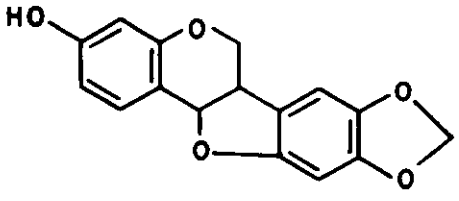
Biochemistry Department, Kuwait University

Kuwait

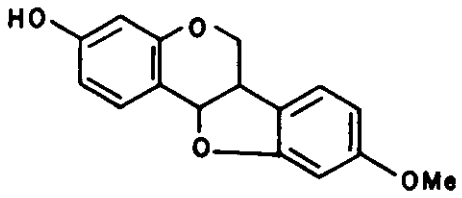
Abstract: Biosynthesis of isoflavonoid phytoalexins and related compounds is reviewed.

Low molecular weight antibiotics "phytoalexins" are produced following interactions between hypersensitive plant tissues and various parasites[1-3]. 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 resistance[4-9]. Reports contrary to this have also appeared claiming that phytoalexins have no role in plant defense mechanisms against fungal infection[10]. Phytoalexins are produced in the first few hours of the penetration of the parasitic cells into the invaded tissues[11-13]. Invasions of the plant by viruses[12,14] or bacteria[15-18] also elicite phytoalexins.

The production of these compounds may also be triggered by abiotic treatment[19-30] of the plant by various factors such as heavy metals[31-53], polysaccharides[54-88], peptides/proteins[87-94], glycoproteins[95-107], metabolic inhibitors[31,108], plant growth substances[109,110], oxidizing reducing agents[108], antimetabolites[111], DNA interchelating agents[112], RNA synthesis[112-114] inhibitors, irradiation with ultraviolet light[115-119], mechanical injury[120,121] and many other factors[122-123]. Biotic and abiotic elicitors act through different mechanisms[134,135]. Thus fenugreek (Trigonella foenum-graecum) leaves on fungal infection produce maakian (1) and



(1)

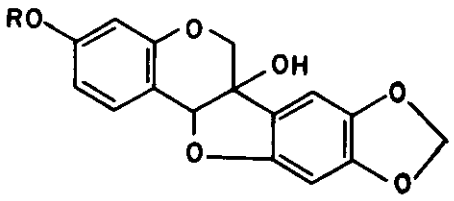


(2)

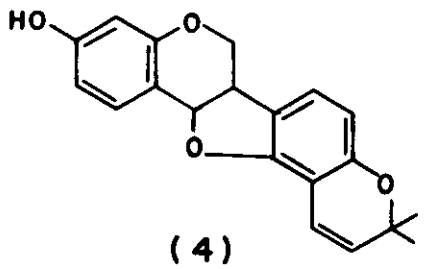
medicarpin (2) in equal amounts, but $CuCl_2$ and UV-treated seedlings produce only maackiain (1) and no medicarpin (2). However, Moesta et al.[36] and Bailey[121] have concluded that biotic and abiotic elicitors act through the same mechanism. The genetic information for phytoalexin production is carried by the host and elicitors of such production, whether specific or non-specific, act through the host genume. 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 isoflavoids[137-139], 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 biosynthesis[147]. Hadwiger and his co-workers have correlated[118] 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 activity[148], which is an intermediate enzyme in the production of pisatin[149,150] (3a), phaseolin[151,152] (4) and other structurally related phytoalexins[153]. Increased level of pisatin (3a) and PAL activity in Pisum sativum treated with antihistaminic, antiviral, antimalarial and



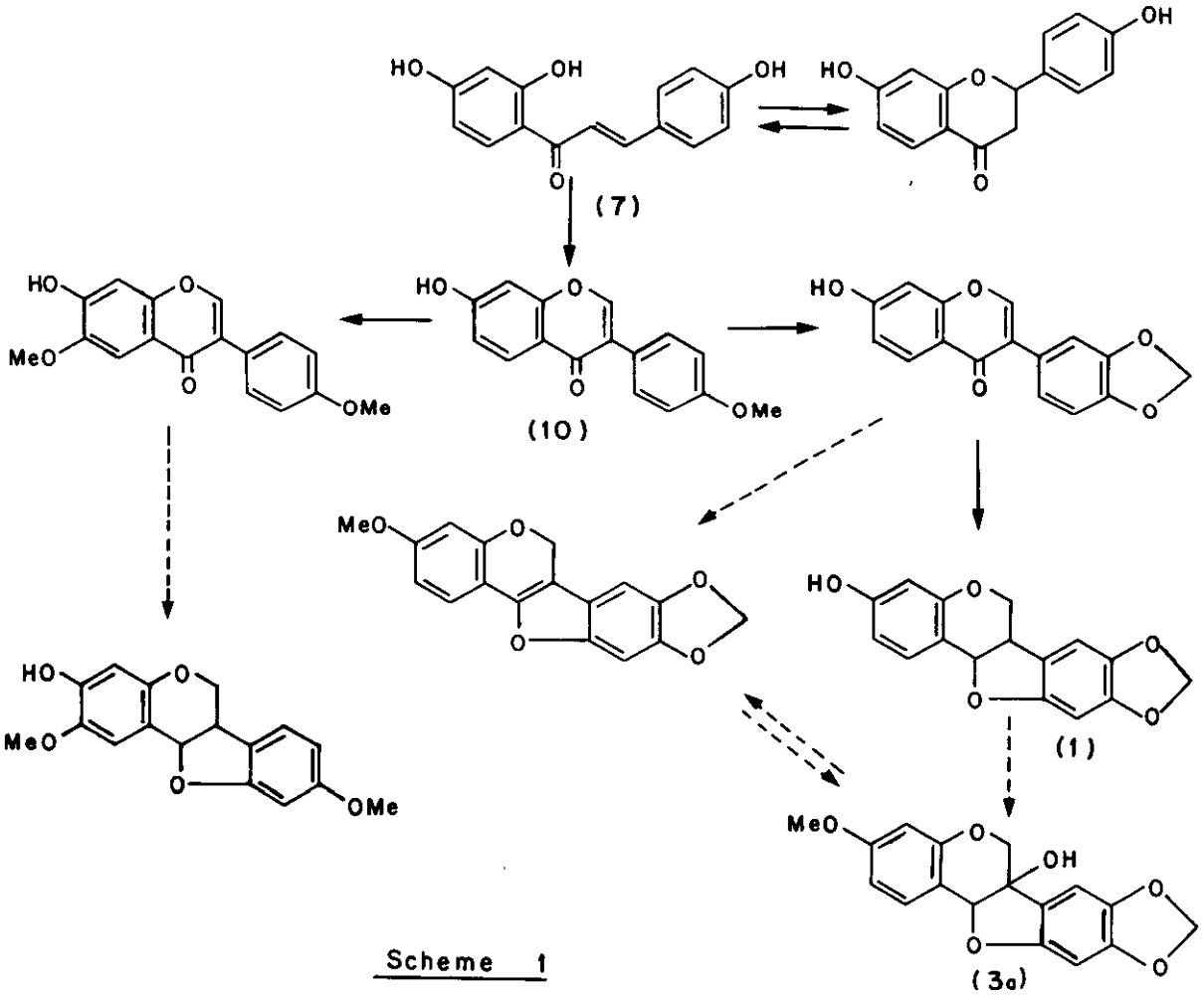
(3a): R = Me
(3b): R = H



(4)

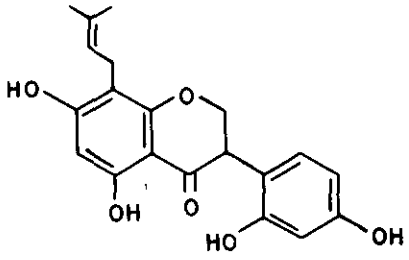
tranquilizing agents has also been reported[154]. Changes in PAL activity have been frequently observed in host-pathogen interactions[35,155,156] and in response to light[157], chemical[155] induction, wounding[158,159] and stress conditions in various plants[160-164].

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 Zucker[165] 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 phytoalexins[166-168] and innermin (3b) has been suggested[167] to be a precursor of pisatin (3a). Biosynthesis of pisatin and other flavonoid phytoalexins in Pisum sativum has been suggested by Carlson and Dolphin[169], as in Scheme 1.



Scheme 1

Although PAL is reported to catalyse the first step in the synthesis of medicarpin[170] (2), it has been suggested that its stimulation may not be a key step in regulation of medicarpin biosynthesis. Similarly Dixon and Fuller[171] have shown that suspension cultures of Phaseollus 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-D[172] producing kievitone (5) and in pea endocrap tissues treated with poly-L-arginine[93]. Patridge and Keen[159] 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-



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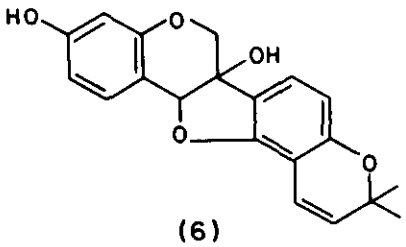
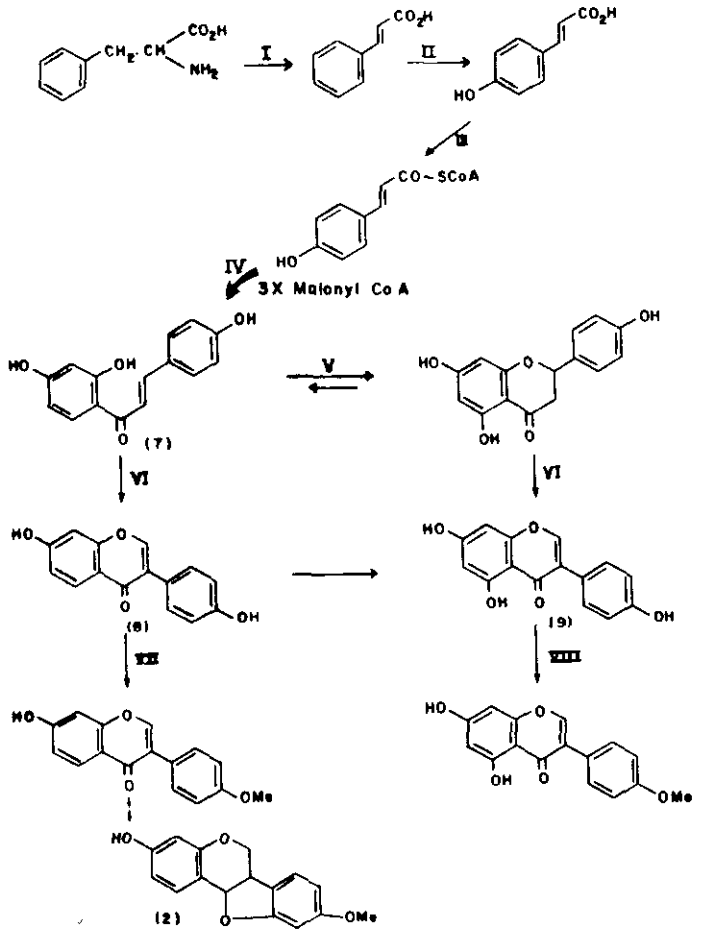


(6)

degratation of 6a-hydroxyphaseolin (6) by soybean hypocotyls infected with Phytophthora megasperma var sojae, have concluded that PAL may in fact be linked with glyceollin biosynthesis. Some other enzymes, apart from PAL, involved in the biosynthesis of isoflavonoids include hydroxycinnamate-CoA ligase, cinnamic acid-4-dydroxylase 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 misundertood as flavone synthetase[241]. Coversion

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 reactions[177-179]. 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 innoculated with Pithomyces chartarum[170] is outlined in scheme 2.



SCHEME 2

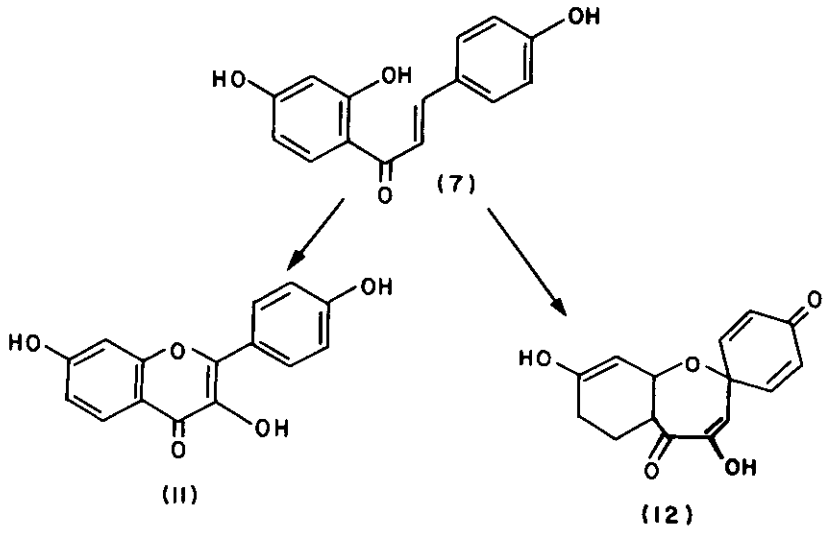
I. PAL *II.* *cinnamic acid-4-hydroxylase*

III. *p-coumaric CoA ligase* *IV.* *chalcone synthetase*

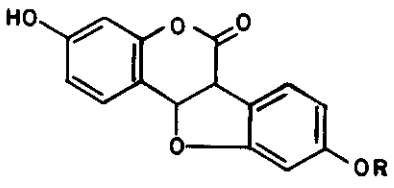
V. *chalcone isomerase* *VI.* *aryl migration*

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).



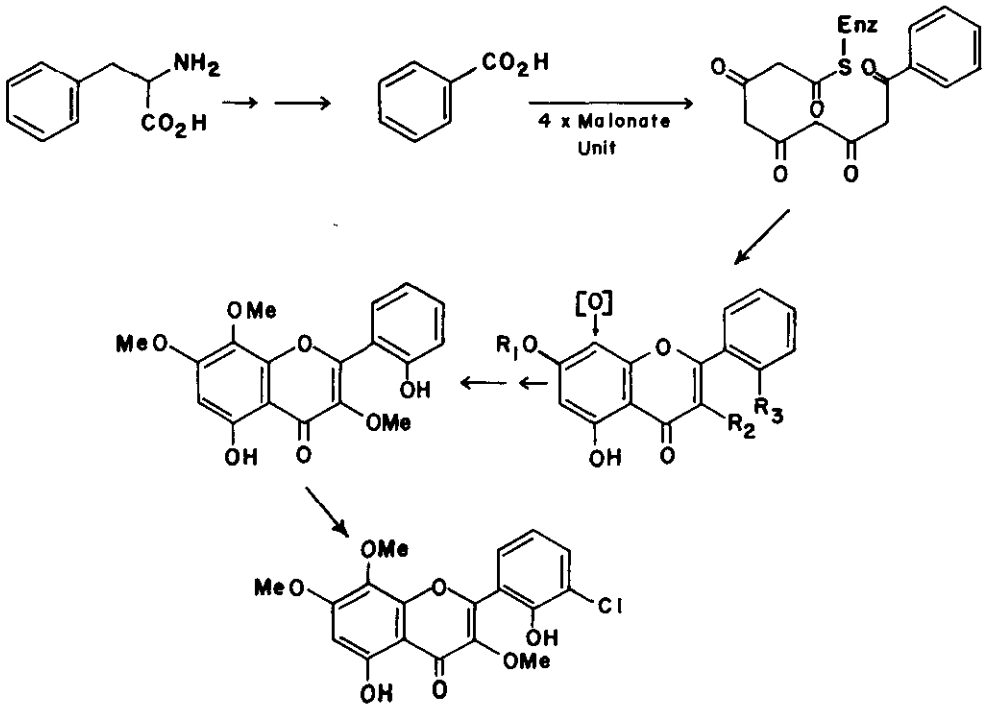
Slight structural variations may result in different controls in the biosynthesis of flavonoid/isoflavonoid phytoalexins. Thus Dixon and Bendall[129] have suggested a separate control for the synthesis of 5-hydroxy- and 5-deoxyflavonoid/isoflavonoid derivatives. These workers have suggested the presence 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-A[129].



(13) : R = H

(14) : R = Me

Flavonoid secondary metabolites are ubliquitous in the plant kingdom and much known about the route by which they are synthesized. However Vinning and McInnes et al[243] have recently reported a pathway of flavonoid biosynthesis differing from that of higher plants in that a C_6-C_1 precursor unit is condensed with four C_2 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.



(14 a)

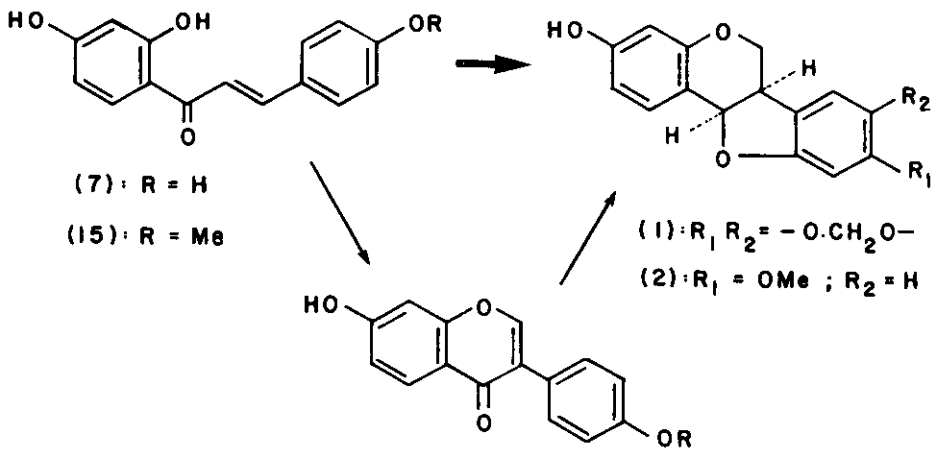
Scheme 3

Several views, for the migration of aryl ring in the isoflavonoid biosynthesis, have been presented. Dewick[186] 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-(^{14}C-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 reported[188]. 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 chickpea[189].

Dewick and Martin[190] have proposed that 4'-hydroxyisoflavones, which are supposed to be derived from proton catalysed decomposition of the postulated spirodienone intermediate[191], are not obligatory intermediates in the biosynthesis of 4'-methoxyisoflavones. These could arise by S-adenosylmethionine mediated decomposition of the spirodienone[177]. However an enzyme catalysing 4'-methylation of daidzein (8) and genistein (9) has been reported[189]. This is regarded as a minor route to the biosynthesis of formonometin[190].

Feeding experiments in $CuCl_2$ treated red clover seedlings have demonstrated that isoliquiritigenin (7) and formononetin (10) are readily incorporated into the pterocarpan phytoalexins 6aR, 11aR-demethylhomopterocarpin[192] (2) and 6aR, 11aR-maackian (1). But 2,4-dihydroxy-4'-methoxychalcone (15) and daidzein (8) were poor precursors[186]. The same four labelled compounds (7,8,10 & 15) have been examined as precursors of medicarpin (2), vestitol (22) and sativan (23) in Medicago sativa[190]. 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), medicarpin[186] (2), maackian[186] (1) and rotenoid amorphigenin[177]. However daidzein (8) and methoxychalcone (15) are also poor precursors for maakian (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 vestitol[199] (22). This has suggested a metabolic grid in Medicago sativa[21,190,200]. Thus isoflav-3-ene type intermediate[201-204]

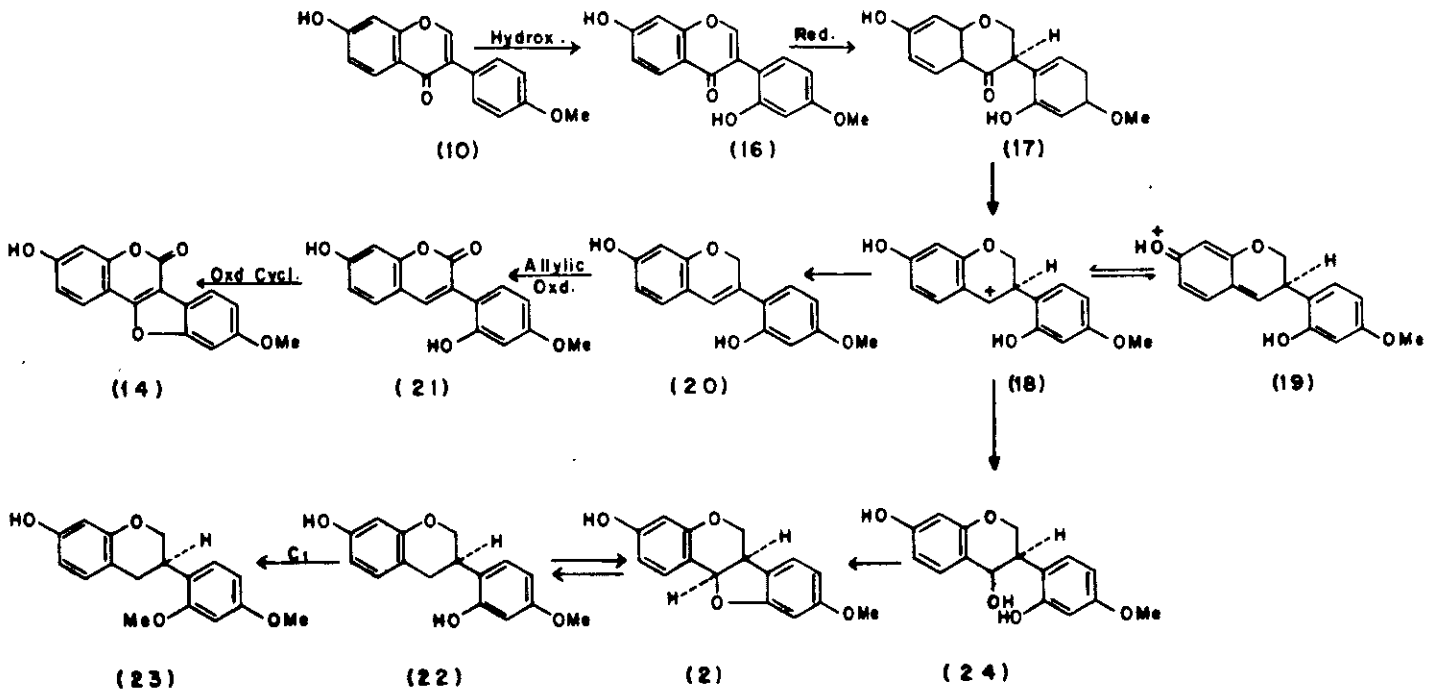


compounds are not important in the biosynthesis of M. staiva phytoalexins or as intermediates in the pterocarpan-2'-hydroxyisoflavan interconversion[199]. On the other hand isoflav-3-ene type compounds are reported to play an important role in coumestans biosynthesis[199].

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 sequence[192] from 2',7-dihydroxy-4'-methoxyisoflavanone (17) via 2',7-dihydroxy-4'-methoxyisoflavone, (16) as reported earlier, in feeding experiments[179]. 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'-hydroxyisoflavans[207]. Feeding experiments in red clover (Trifloium 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 medicarpin[179].

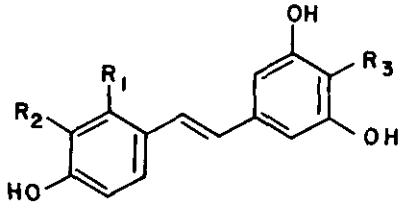
Reductive ring opening of a pterocarpan to a 2'-hydroxyisoflavan[208-211], and methylation of an isoflavonoid are among the demonstrated metabolic processes initiated by fungi[116,212]. Biochemically, the pterocarpan ⟶ 2'-hydroxyisoflavan conversion has been demonstrated during fungal detoxication of pterocarpan phytoalexins, such as maackiain (1), medicarpin (2) and phaseollin[209-211] (4). Red clover is reported to synthesize only pterocarpan phytoalexins producing


Hydrox.
Red.
Oxd Cycl.
Allylic Oxd.
Cl
(10)
(16)
(17)
(14)
(21)
(20)
(18)
(19)
(23)
(22)
(2)
(24)

Scheme 4

maackiain (1) and medicarpin[213]. 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 pterocarpans[214]. Exception to this has been reported by Dewick[179] 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 incuced with abiotic treatment have been isolated and characterized. These compounds are all trans-resveratrol analogues (25-30), accumulated in Grapvine[215,216], Arachis hypogaea[217-219] 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

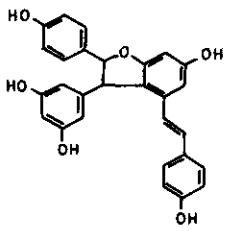
27: 2-Hydroxyresveratrol: $R_2=R_3=H$; $R_1=OH$

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 predicted[215] as a biosynthetic precursor of the viniferins ξ (31) and α (32), antifungal compounds characteristic of the family Vitaceae.



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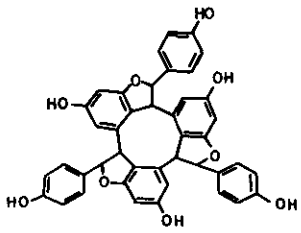
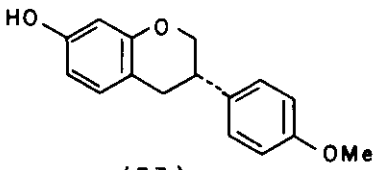


(32)

The co-occurrence of isoflavan broussin (33) and bisarylpropanoid broussin-C (34) in mulberry (Broussonetia papyrifera vent.) has suggested[221] 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 G[223] (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 hypothesis[224].



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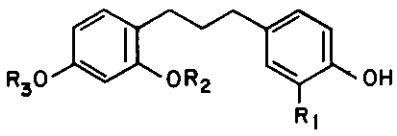
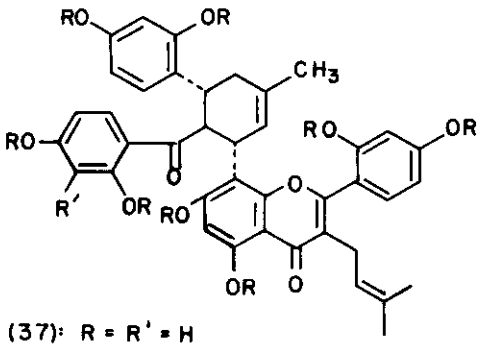
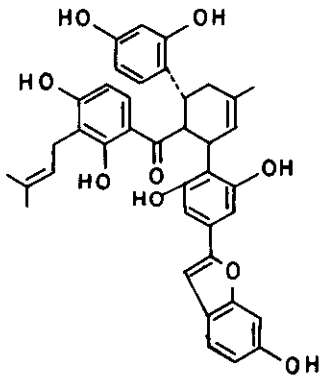


(34): $R_2 = R_3 = H$; R_1 = isopentenyl
(35): $R_1 = R_2 = H$; R_3 = Me
(36): $R_1 = R_3 = H$; R_2 = Me



(37): R = R' = H
(38): R = H, R' = prenyl



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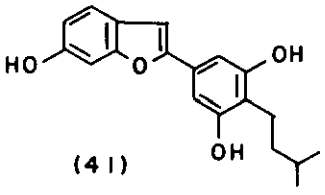

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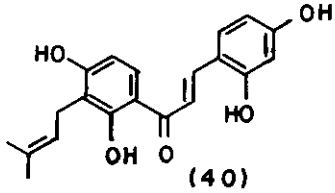

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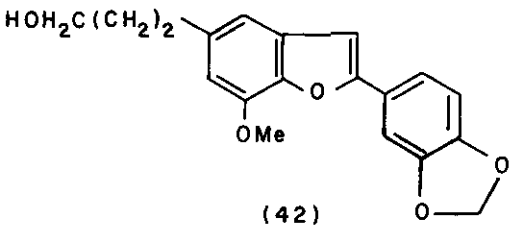

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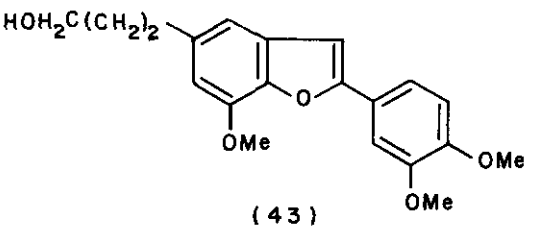

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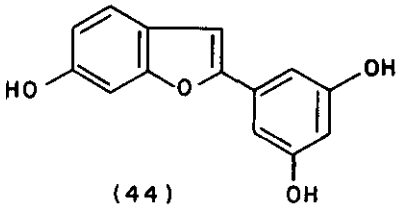

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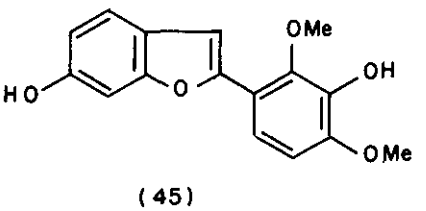
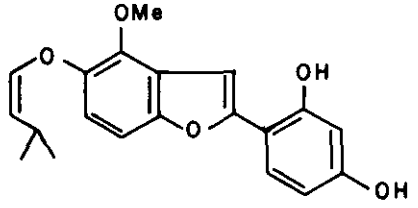


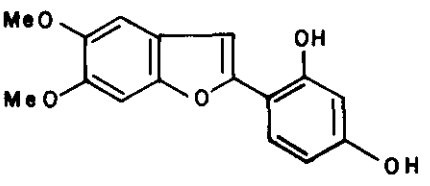
(45)

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 arylbenzofurans[242]. Thus Martin and Dewick have suggested[242] that egonol (42) and homoegonol (43), from styrax species[228-230] are formed from bisarylpropanoids by loss of a carbon atom.

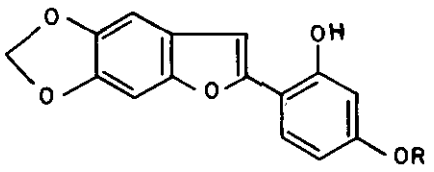
However if a cyclization process preceeds a reduction of chalcone, a flavone may result



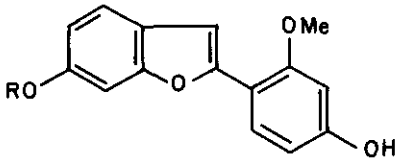
(46)



(47)

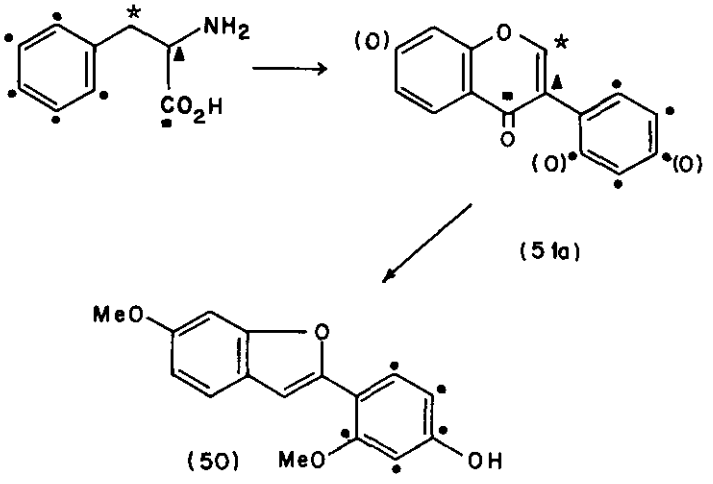


(48) : R = H
(49) : R = Me



(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 coumastan, during biosynthesis of vignafuran has also been reported[234,239]. Pterofuran (45) from Pterocarpus indicus[232], neoraufurane (46) from Neorautanenia 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 atom[242].

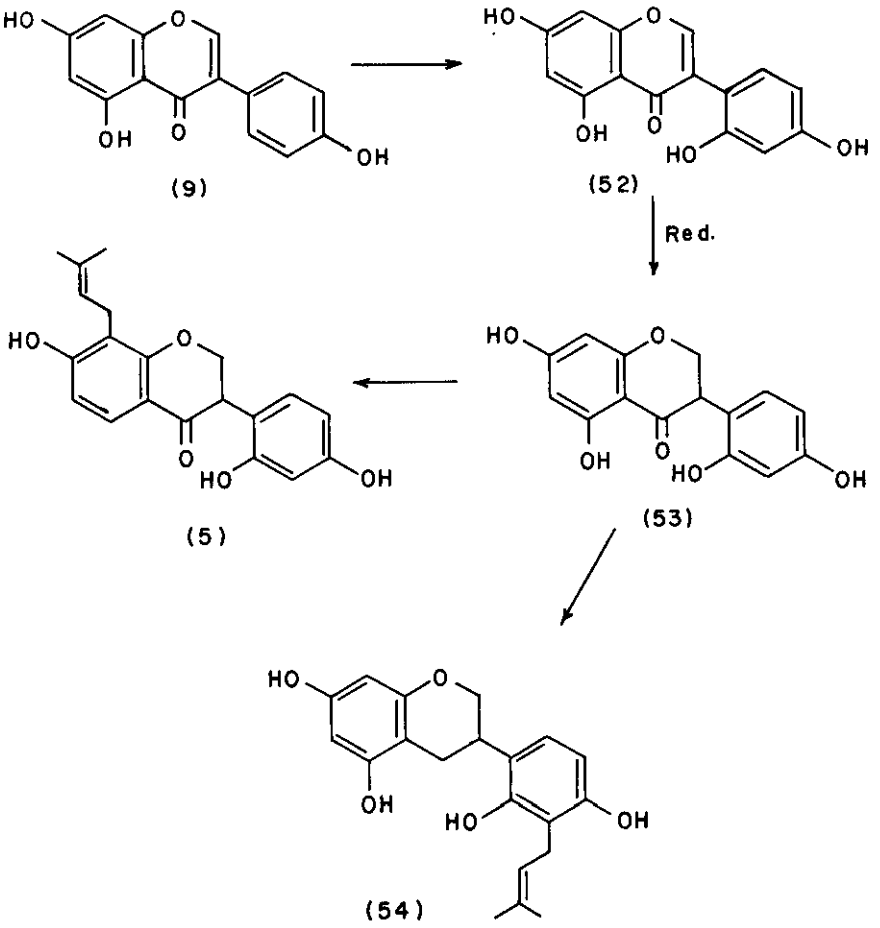


(51a)

(50)

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 proposed[240] 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 suggested[240] 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.



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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