Biosynthesis of the Rotenoid Amorphigenin in Germinating *Amorpha fruticosa* Seeds: the Pre-rotenoid (Isoflavonoid) Stages

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Summary. Insertion of the rotenoid 'extra' C-6 methylene occurs at the isoflavone (IIIa), rather than the isoflavanone (IVa), level of oxidation, in the biosynthesis of amorphigenin; earlier stages use chalcone (Va) with a free 4 hydroxy-group, but the biosynthetically acceptable isoflavone which emerges is not (VIa) but its methyl ether (VIb).

9-DEMETHYLMUNDUSERONE (I) has been characterised as a pivotal intermediate in the biosynthesis of amorphigenin (II) in *Amorpha fruticosa* seeds.[1] It is formed from the isoflavone (IIIa), the 2′-methoxy-group providing the 'extra' C-6 of the rotenoid.[1,2] The possibility remains, however, that the isoflavanone (IVa) could be the actual intermediate in the ring-B cyclisation. Accordingly, 7-

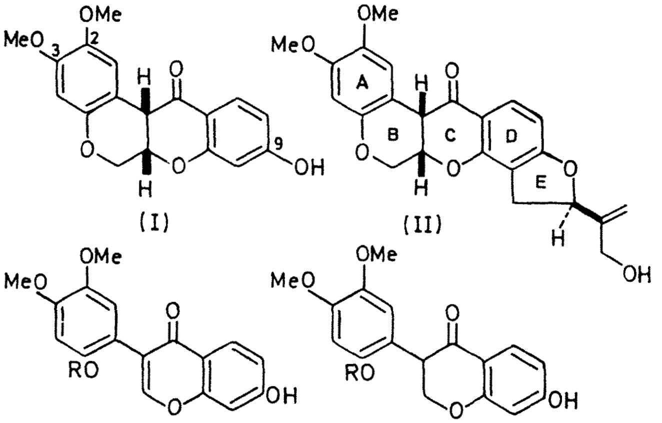
TABLE 1

Incorporation of isoflavone (IIIb) *and isoflavanone* (IVb) *into amorphigenin by germinating* A. fruticosa *seeds*

Feeding period (h)	Isoflavone (IIIb)[a] Incorporation (%)[b]	Dilution	Uptake (%)	(±)-Isoflavanone (IVb)[a] Incorporation (%)[b]	Dilution	Uptake (%)
6	0·049	—	56	0·016	—	40
12	0·057	2300	63	0·020	6100	54
24	0·19	1130	57	0·024	8900	50
48	0·84	470	66	0·045	7280	59

[a] Sodium salt, phosphate buffer, pH 7·0.
[b] Arbitrary correction of 1/3 made for tritium loss on incorporation.

hydroxy-$[2'-{}^3H]$-methoxy-4',5'-dimethoxyisoflavone (IIIb) and (±)-7-hydroxy-$[2'-{}^3H]$-methoxy-4',5'-dimethoxyisoflavanone (IVb) were compared in parallel experiments (cancellation of tritium isotope effect) by administration to *A. fruticosa* germinating seeds. A series of time periods was used. Results (Table 1) show that the isoflavone (IIIb) is used considerably more efficiently than the isoflavanone (IVb) (correction for expected involvement of only one enantiomorph of the latter being taken in account.) In support, (IIIa) can be isolated from the *A. fruticosa* seed system by isotope dilution.[1]

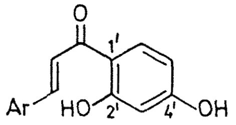


(I)

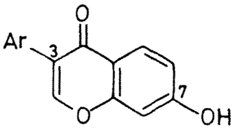
(II)

(IIIa) R = CH_3
(IIIb) R = CT_3
(IIIc) R = $^{14}CH_3$

(IVa) R = CH_3
(IVb) R = CT_3



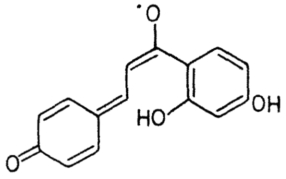
(V)



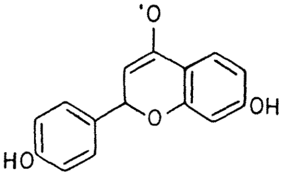
(VI)

(a) Ar = $C_6H_4 \cdot p$-OH
(b) Ar = $C_6H_4 \cdot p$-OMe
(c) Ar = C_6H_4-2,4-$(OH)_2$

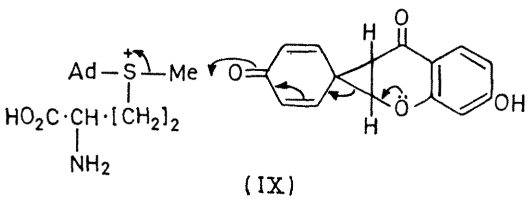
(d) Ar = C_6H_3-3,4-$(OH)_2$
(e) Ar = C_6H_3-3,4-$(OMe)_2$
(f) Ar = C_6H_2-2,4,5-$(OMe)_3$



(VII)



(VIII)



(IX)

Phenyl migration from the original C-3 of phenylalanine (or cinnamic acid) to C-2 during rotenoid biosynthesis was the first experimental evidence for the isoflavonoid pattern of biosynthesis of the rotenoids.[2,3] Such precursors are elaborated to chalcones, which may equilibrate with flavanones,[4] and in order to obtain further details on the aryl migration a series of ^{14}C-labelled chalcones was administered to *A. fruticosa*. Results (Table 2) show that from a series of mono-, di-, and tri-oxygenated ring-A chalcones, much the best incorporation was found for 2',4,4'-trihydroxychalcone (Va). Such a result is in line with Grisebach's work in which it was shown that a 4-methoxylated cinnamic acid destined for isoflavonoid biosynthesis becomes demethylated.[5] A number of hypotheses have been proposed for the mechanism of the

TABLE 2

Incorporation of chalcones and isoflavones into amorphigenin by germinating A. fruticosa *seeds*[a]

		Incorporation (%)	Dilution	Uptake (%)
Series A				
Chalcone (Va)[b]	..	0·30	1080	87
» (Vb)[b]	..	0·006	44,000	86
» (Vd)[b]	..	0·019	13,900	81
» (Ve)[b]	..	0·002	19,500	43
» (Vf)[b]	..	0·003	70,200	78
Isoflavone (VIb)[d]	..	0·42	515	83
» (IIIc)[c]	..	1·81	158	70
Series B				
Chalcone (Va)[b]	..	1·22	732	89
Isoflavone (VIa)[b]	..	0·013	94,600	80
» (VIc)[e]	..	0·002	743,000	89
» (IIIc)[c]	..	8·70	150	78

[a] Compounds administered as sodium salts in phosphate buffer, pH 7·0, over 48 h.

[b] [Carbonyl—^{14}C].

[c] [2'—O^{14}CH$_3$].

[d] [4'—O^{14}CH$_3$].

[e] Tritiated by Wilzbach method, followed by removal of alkaline-exchangeable tritium: less than 3% of tritium is at C-2.

isoflavonoid aryl migration, and *in vitro* chemical analogies attempted.[6] The proposal of Pelter[7] assigns a role to the 4-hydroxy-group of the migrating aryl, is chemically acceptable, and appears to accommodate such requirements as are currently recognised. One-electron transfer leads from chalcone (Va, anion) *via* (VII) to (VIII). A second one-electron transfer gives a spirodienone (*cf.* IX).

The product of proton-catalysed decomposition of the spiro-dienone, 4',7-dihydroxy-isoflavone (VIa, daidzein) is not, however, an acceptable precursor for amorphigenin (Table 2). On the other hand, 7-hydroxy-4'-methoxyisoflavone (VIb, formononetin)† is very satisfactorily incorporated and this situation is explained if the spirodienone is decomposed by methylation, *e.g.* by *S*-adenosylmethionine, (IX) followed by proton loss. Hydroxylation and *O*-methylation, in undetermined manner, are presumed to convert (VIb) into the precursor (IIIa) for forming rotenoid (I).

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† Daidzein (VIa) is poorly incorporated into formononetin (VIb) in *Cicer arietinum* or *Medicago sativa*, although it is converted into coumestrol in the latter plant.[8]

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