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ROLE OF AN ISOFLAV-3-ENE IN THE BIOSYNTHESIS OF PTEROCARPAN, ISOFLAVAN AND COUMESTAN METABOLITES OF MEDICAGO SATIVA

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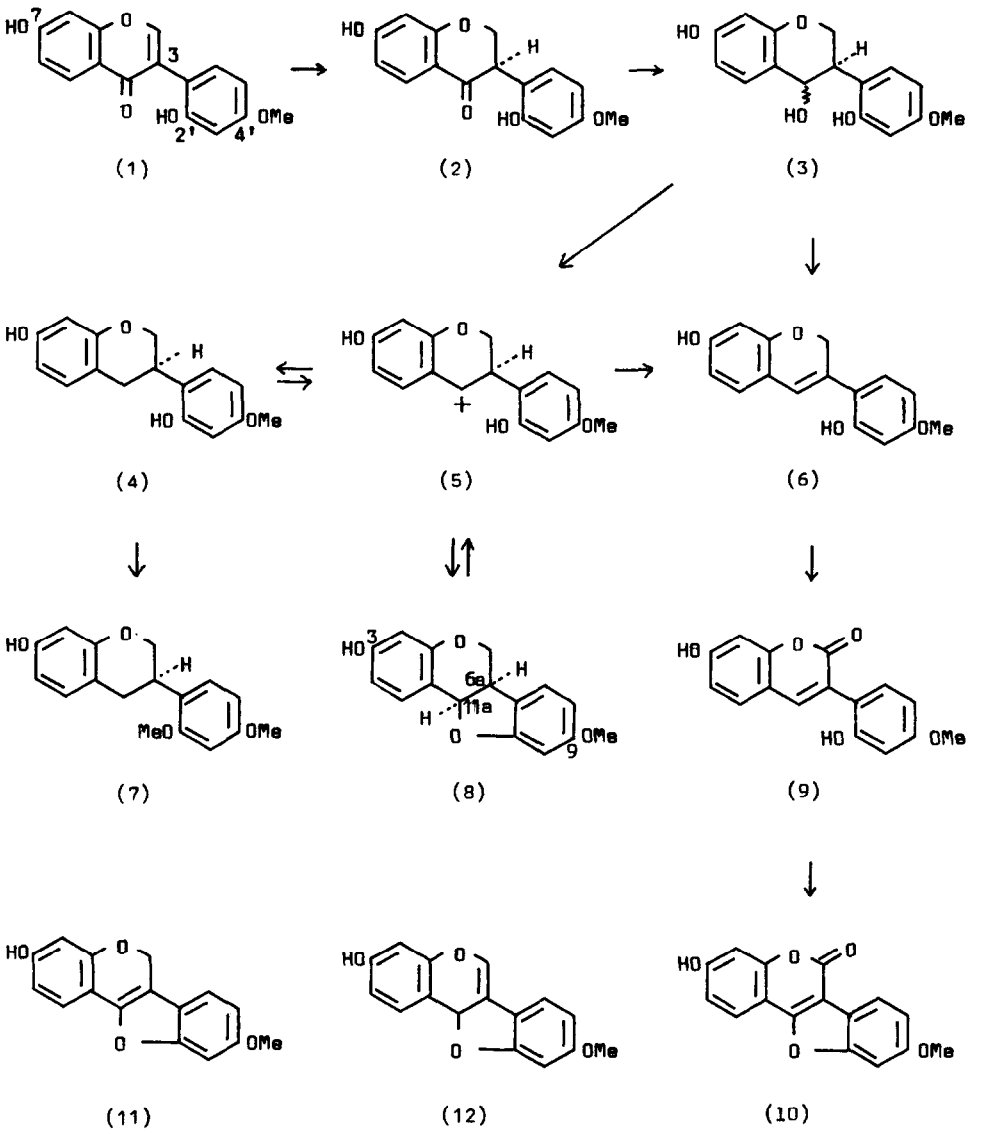
Recent reports[1,2,3] concerning the isolation of isoflav-3-enes from leguminous plants have increased speculation[2,4] that these compounds may play a role in the biosynthesis of other isoflavonoids. In particular, 2',7-dihydroxy-4'-methoxyisoflav-3-ene (6) has been considered[5] as an intermediate in the biosynthesis of the phytoalexins (6aR, 11aR)-demethylhomopterocarpin (8), (3R)-vestitol (4) and (3R)-sativan (7) in lucerne (Medicago sativa). These compounds are derived by a stereospecific reductive sequence[6] from 2',7-dihydroxy-4'-methoxyisoflavone (1) via 2',7-dihydroxy-4'-methoxyisoflavanone (2) and probably the corresponding isoflavanol (3). Compounds (4) and (8) are produced simultaneously, but are readily interconvertible in M. sativa, and an intermediate carbonium ion (5) has been proposed[5]. However, isoflav-3-ene (6) could be an alternative uncharged intermediate. Sativan (7) appears to be derived by methylation of vestitol(4).

To test the role of this isoflav-3-ene in the interconversion of (4) and (8), (±)-demethylhomopterocarpin-[6a-^{3}H,9-OMe-^{14}C] and (±)-vestitol-[3-^{3}H,4'-OMe-^{14}C] were fed to UV-($\frac{1}{2}$ hr) and $CuCl_2$-(8 hr) treated seedlings of M. sativa. After a metabolism period of 16 hr, the phytoalexins (4),(7) and (8) were isolated, together with 9-O-methylcoumestrol (10), which, although not fungitoxic, is synthesised in increased amounts after this abiotic stimulation. The results, Table 1, show that demethylhomopterocarpin was transformed into

Table 1. Incorporation of doubly-labelled demethylhomopterocarpin and vestitol into M. sativa metabolites

Compound fed / Metabolite	(±)-Demethylhomopterocarpin-[6a-^{3}H,9-OMe-^{14}C] (^{3}H/^{14}C = 5·0)		(±)-Vestitol-[3-^{3}H,4'-OMe-^{14}C] (^{3}H/^{14}C = 5·0)	
	^{3}H/^{14}C	% Incorp	^{3}H/^{14}C	% Incorp
Demethylhomopterocarpin	5·5	39	5·0	1·4
Vestitol	5·1	0·93	5·3	7·1
Sativan	5·2	0·97	5·2	0·83
9-O-methylcoumestrol	0·1	0·21	0·1	0·04



vestitol and sativan with essentially no change in the $^{3}H/^{14}C$ ratio, but virtually all the ^{3}H was lost on incorporation into the coumestan. Similarly, vestitol was converted into demethylhomopterocarpin and sativan without significant change in the $^{3}H/^{14}C$ ratio; incorporation into the coumestan was very small by comparison, but all ^{3}H was again lost. These data prove that an isoflav-3-ene cannot be involved in the pterocarpan-2'-hydroxyisoflavan interconversion, since such an intermediate would necessitate complete loss of the ^{3}H label. Chemical analogies[7] for the interconversion similarly favour a carbonium ion intermediate rather than an isoflav-3-ene.

In a further series of comparative feeding experiments, the [Me-^{14}C]-labelled

isoflavonoids, 2',7-dihydroxy-4'-methoxyisoflavone (1), (±)-demethylhomopterocarpin (8), 2',7-dihydroxy-4'-methoxyisoflav-3-ene (6) and the 3-phenylcoumarin, 2',7-dihydroxy-4'-methoxyisoflav-3-ene-2-one (9) were administered. The results are shown in Table 2. Isoflavone (1) was an excellent precursor of all compounds, in agreement with earlier observations[5,8], and pterocarpan (8) was converted into both isoflavans and the coumestan

Table 2. Incorporation[‡] of [Me-^{14}C]-labelled isoflavonoids into *M. sativa* metabolites

Compound fed \ Metabolite	Demethyl-homopterocarpin	Vestitol	Sativan	9-O-methyl-coumestrol
Isoflavone (1)	7·9 (18)	1·0 (16)	0·34 (24)	0·66 (11)
(±)-Pterocarpan (8)	33 (3·9)	0·13 (98)	0·41 (32)	0·31 (25)
Isoflav-3-ene (6)	0·09 (1240)	0·04 (390)	0·006 (2750)	5·3 (2·1)
Phenylcoumarin (9)	0·06 (1840)	0·02 (680)	0·007 (2820)	2·2 (3·8)

‡ Incorporation expressed as percentage, figures in brackets are dilution values.

(cf Table 1). However, the isoflav-3-ene (6) was a very poor precursor of (8), (4) and (7), further suggesting that this compound is not involved in pterocarpan and isoflavan biosynthesis. In contrast, it proved an extremely efficient precursor of the coumestan (10), as also was the phenylcoumarin (9).

Earlier experiments[9,10] have suggested that the biosynthetic route to coumestans may involve allylic oxidation of a pterocarp-6a-ene, e.g. (11), produced by dehydration of a 2'-hydroxyisoflavanone, e.g. (2). Isoflavanone (2) is an excellent precursor of (10) in *M. sativa*[8]. Since pterocarp-6a-enes are so readily susceptible to autoxidation yielding coumestans[11,12,13], they have not been tested as precursors in feeding experiments. In the light of the above results, this route seems less likely than a pathway involving dehydration of the isoflavanol (3) to isoflav-3-ene (6), allylic oxidation and then ring cyclisation by attack of the 2'-hydroxy onto the α,β-unsaturated lactone followed by further oxidation. Isoflav-3-ene (6) may, however, also be derived by loss of a proton from carbonium ion (5), and such a route would explain the significant incorporation of pterocarpan (8) into the coumestan.

A chemical analogy for the isoflav-3-ene -coumestan conversion is provided by the reaction of (6) with DDQ in dioxan at R°, which produced (10) in 84% yield. Only traces of coumestan were observed on similar treatment of (9) however, but a yield of 18% was obtained by heating with DDQ in refluxing benzene over 16 hr. Similar yields of coumestans have been obtained by treating 2'-hydroxyisoflav-3-ene-2-ones with lead tetracetate[14]. Probably then the facile DDQ oxidation of (6) to (10) does not parallel the biosynthetic pathway, and may involve the formation of an extended quinonemethide and cyclisation of the 2'-hydroxy onto this system, producing pterocarp-6-ene (12) as an intermediate. Further oxidation of (12) would yield the coumestan[15]. Although isoflav-3-ene (6) is very reactive, especially in solution, our studies have shown that over 24 hr, coumestan (10) is not formed as an

autoxidation product; in addition, no coumestan is formed on atmospheric oxidation of phenylcoumarin (9).

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