# Biosynthesis of Isoflavonoid Phytoalexins in *Medicago sativa*: the Biosynthetic Relationship between Pterocarpans and 2′-Hydroxyisoflavans

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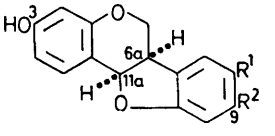
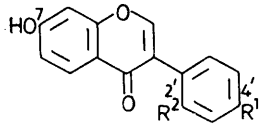
*Summary* Demethylhomopterocarpin (**1**) and vestitol (**8**) are interconvertible in $CuCl_2$-treated lucerne seedings, but they appear to be synthesised simultaneously from a common intermediate; sativan (**9**) is probably derived by the methylation of vestitol.

HYDROGENOLYSIS of pterocarpans readily yields 2′-hydroxyisoflavans,[1] and this type of reductive process has been postulated to occur[2] during isoflavan biosynthesis in plants. Certain fungi are known to transform pterocarpan phytoalexins to 2′-hydroxyisoflavans as part of a detoxification sequence.[3] On the other hand, it has been suggested[4] that pterocarpans may be produced by oxidation of 2′-hydroxyisoflavans. Feeding experiments in red clover (*Trifolium pratense*)[5,6] have suggested that the biosynthetic pathway to (6a*R*,11a*R*)-demethylhomopterocarpin (**1**), proceeds *via* the isoflavone (**3**), followed by 2′-hydroxylation to (**4**), and reduction to the isoflavanone (**6**). This isoflavanone is probably reduced further to the isoflavanol (**7**) which can then cyclise to (**1**). The biological reduction sequence is presumably stereospecific.

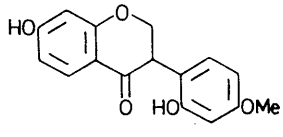
On fungal infection,[7] lucerne (*Medicago sativa*) produces three phytoalexins, (6a*R*,11a*R*)-demethylhomopterocarpin (**1**), (3*R*)-sativan (**9**), and vestitol (**8**).† Synthesis of the same compounds can be induced by treatment of lucerne seedlings with $Cu^{2+}$ ions.[8]‡ Labelled isoflavonoids were tested in comparative feeding experiments as precursors of (**1**), (**8**), and (**9**) in $CuCl_2$-treated seedlings, and the results are summarised in the Table. Compound (**1**) was purified as its methyl ether,[5] while (**8**) and (**9**) were obtained as their acetates.



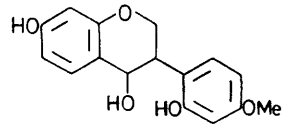
(1) $R^1$=H, $R^2$=OMe
(2) $R^1R^2$=O-$CH_2$-O



(3) $R^1$=OMe, $R^2$=H
(4) $R^1$=OMe, $R^2$=OH
(5) $R^1$=$R^2$=OMe



(6)



(7)

† The absolute configuration is believed to be 3*R*.

‡ In our experiments, even aseptically-grown seedlings produced small amounts of these phytoalexins, but treatment with $CuCl_2$ greatly stimulated the synthesis. Typically, (**1**), (**9**), and (**8**) were produced in a ratio of about 6 : 2 : 1, 24 h after induction.

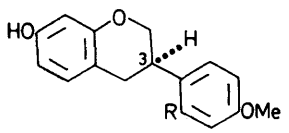
[*Me*-$^{14}$C]-2′,7-Dihydroxy-4′-methoxyisoflavone (**4**) and (±)-[*Me*-$^{14}$C]-2′7-dihydroxy-4′-methoxyisoflavanone (**6**) were excellent precursors of demethylhomopterocarpin[6] and both isoflavans. In contrast, [4′-*Me*-$^{14}$C]-7-hydroxy-2′,4′-dimethoxyisoflavone (**5**), a possible precursor of (**10**) or its mesomeric counterpart (**11**) derived[9] from the isoflavanol (**7**). Cyclisation and loss of a proton would lead to the pterocarpan, whilst reduction–addition of a hydride ion would produce the isoflavan. Structure (**11**) represents the protonated form of the quinonemethide

TABLE. Incorporation[a] of [*Me*-$^{14}$C]-labelled compounds into phytoalexins in lucerne

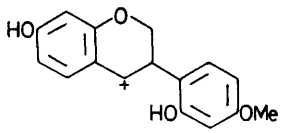
| Compound fed[b] | Demethylhomopterocarpin | | Vestitol | | Sativan | |
|---|---|---|---|---|---|---|
| | Incorporation/% | Dilution | Incorporation/% | Dilution | Incorporation/% | Dilution |
| Isoflavone (**4**) .. .. .. | 10·1 | 9·9 | 0·82 | 21 | 0·63 | 35 |
| Isoflavone (**5**) .. .. .. | 0·024 | 4700 | 0·0035 | 7000 | 0·0023 | 16000 |
| (±)-Isoflavanone (**6**) .. .. | 4·1 | 20 | 0·31 | 38 | 0·58 | 50 |
| (±)-Pterocarpan (**1**) .. .. | 39·0 | 4·1 | 0·31 | 40 | 0·58 | 45 |
| (±)-Isoflavan (**8**) .. .. .. | 0·21 | 120 | 14·9 | 2·0 | 0·25 | 49 |

[a] Data from feedings of racemates are uncorrected for the possible utilisation of only one enantiomer. Compounds administered as sodium salts in phosphate buffer, pH 7·0, 8 h after $CuCl_2$ inducer was added. Feeding period was 16 h.

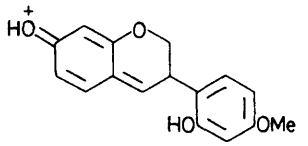
sativan, was poorly utilised. (±)-[*Me*-$^{14}$C]-Demethylhomopterocarpin (**1**) was significantly incorporated into vestitol and sativan, as was (±)-[*Me*-$^{14}$C]-vestitol (**8**) into sativan and demethylhomopterocarpin.



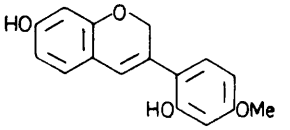

(**8**) R = OH
(**9**) R = OMe




(**10**)




(**11**)




(**12**)

In lucerne, therefore, demethylhomopterocarpin and vestitol are interconvertible. However, the incorporation figures suggest that neither transformation is the normal route to these compounds. The results imply the existence of a common intermediate on the pathway to demethylhomopterocarpin and vestitol, which are probably synthesised simultaneously. Reversal of these pathways back to this intermediate would explain the interconversion. Such an intermediate could be the carbonium ion intermediate postulated[4] in the chemical conversion of (**8**) into (**1**). An alternative, uncharged intermediate, not yet excluded by feeding experiments, might be the isoflav-3-ene (**12**). However, if isoflav-3-enes are intermediates in the biosynthesis of pterocarpans and isoflavans, it is surprising that no naturally occurring example of this class of isoflavonoid has been reported yet.

Additional evidence for the existence of a common intermediate is provided by the results of kinetic feeding experiments over 48 h using [*U*-$^{14}$C]-L-phenylalanine. Incorporation curves for demethylhomopterocarpin and vestitol showed similar trends, incorporation reaching a maximum some 6 h after administration, then decreasing fairly quickly. The curve for sativan showed maximum incorporation at about 12 h. Sativan is probably derived by methylation of vestitol. A reductive sequence from 2′,4′-dimethoxyisoflavone (**5**) can be excluded.

(±)-[*Me*-$^{14}$C]-Vestitol (**8**) has also been tested as a precursor of the pterocarpans in red clover. It was incorporated into demethylhomopterocarpin (**1**) (0·90%, dilution 19) but not into (6a*R*,11a*R*)-maackiain (**2**) (0·0018%, dilution 8400). Comparative figures for (±)-[*Me*-$^{14}$C]-2′,7-dihydroxy-4′-methoxyisoflavanone (**6**) were: demethylhomopterocarpin (2·7%, dilution 4·5), maackiain (0·0047%, dilution 3200). These figures also suggest that the pterocarpan-2′-hydroxyisoflavan interconversion is not the normal sequence.

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