Pterocarpan Biosynthesis: 2′-Hydroxy-isoflavone and -isoflavanone Precursors of Demethylhomopterocarpin in Red Clover

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Summary 2′-Hydroxy-isoflavone (**7**) and -isoflavanone (**8**) are excellent precursors of demethylhomopterocarpin (**1**) but not maackiain (**2**) in Cu^{2+}-treated red clover seedlings; pterocarp-6a-ene (**9**) is hardly incorporated into the pterocarpans and (**1**) is not a precursor of (**2**).

SEEDLINGS of red clover (*Trifolium pratense*), on fungal infection[1] or treatment with Cu^{2+} ions,[2] synthesise two phytoalexins, the pterocarpans (6a*R*,11a*R*)-demethylhomopterocarpin (**1**) and (6a*R*,11a*R*)-maackiain (**2**). Recent feeding experiments[2] have demonstrated that the chalcone (**3**) and isoflavone (**6**) are good biosynthetic precursors of both (**1**) and (**2**) in red clover, but the chalcone (**4**) and isoflavone (**5**) are hardly incorporated, results which may be rationalised if methylation is an integral part of the aryl migration process involved in the biosynthesis of isoflavones.[3]

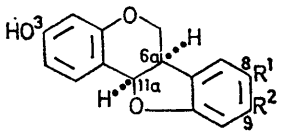
TABLE

Incorporation of [*Me*-^{14}C]-labelled compounds into pterocarpans in red clover

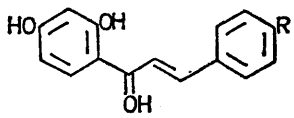
Compound fed[a]	Demethylhomopterocarpin		Maackiain	
	Incorporation (%)	Dilution	Incorporation (%)	Dilution
Isoflavone (**6**)	0·62	34	0·51	59
Isoflavone (**7**)	12·8	2·7	0·0035	7900
(±)-Isoflavanone (**8**) ..	7·6	3·2	0·0019	11900
Pterocarp-6a-ene (**9**) ..	0·040	420	0·0044	5600
(±)-Pterocarpan (**1**) ..	35·6	1·5	0·0035	6100

[a] Compounds administered as sodium salts in phosphate buffer, pH 7·0, 8 h after $CuCl_2$ inducer added. Feeding period 16 h. Uptake of label 70—80%.

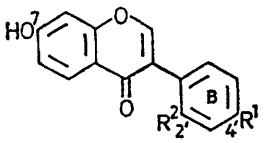
In the present studies, further labelled isoflavonoids have been tested as precursors of (**1**) and (**2**) in $CuCl_2$-treated red clover seedlings. The results of comparative feeding experiments are summarised in the Table, which also includes incorporation data for [*Me*-^{14}C]-7-hydroxy-4′-methoxyisoflavone (**6**). The pterocarpans were purified as their methyl ethers as previously described.[2]



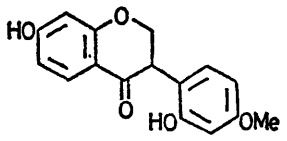
(1) R^1=H, R^2=OMe
(2) R^1R^2=O-CH_2-O



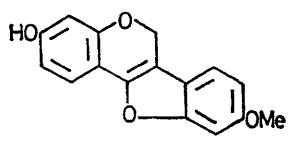
(3) R = OH
(4) R = OMe



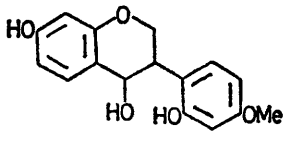
(5) R^1=OH, R^2=H
(6) R^1=OMe, R^2=H
(7) R^1=OMe, R^2=OH



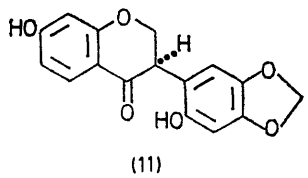
(8)



(9)



(10)



(11)

[*Me*-^{14}C]-2′,7-Dihydroxy-4′-methoxyisoflavone (**7**) and (±)-[*Me*-^{14}C]-2′,7-dihydroxy-4′-methoxyisoflavanone (**8**) are both excellent precursors of demethylhomopterocarpin but not maackiain. The incorporation of [*Me*-^{14}C]-3-hydroxy-9-methoxypterocarp-6a-ene (**9**) into (**1**) was insignificant by comparison however, and into (**2**) was negligible, suggesting that reduction of such compounds to pterocarpans is not a pathway of any importance. The instability of pterocarp-6a-enes towards atmospheric oxygen is well known[4,5] and the initial stages (3·5 h) of feeding experiments utilising (**9**) were conducted in an N_2 atmosphere using deoxygenated solvents, thus minimising loss of precursor by oxidation.† (±)-[*Me*-^{14}C]Demethylhomopterocarpin was not incorporated into maackiain, confirming conclusions reached earlier from feeding experiments with phenylalanine.[2]

A number of workers[4,6] have postulated that pterocarp-6a-enes, formed by dehydration of 2′-hydroxyisoflavanones or oxidative cyclisation of 2′-deoxyisoflavanones, occupy a key position in the biosynthetic pathway to other isoflavonoids, giving for example, pterocarpans by reduction, coumestans by allylic oxidation, and 6a-hydroxypterocarpans by the addition of H_2O. The insignificant incorporation of (**9**) into (**1**) thus necessitates an alternative hypothesis for pterocarpan biosynthesis. From the above results, the most likely route from isoflavone (**6**) to demethylhomopterocarpin involves 2′-hydroxylation to (**7**), reduction to the isoflavanone (**8**) followed probably by further reduction to the isoflavanol (**10**) and subsequent cyclisation.[7] Such a pathway would be analogous to laboratory syntheses of pterocarpans by $NaBH_4$ reduction of 2′-hydroxyisoflavones.[8] The results however, do not exclude the production of (**10**) from (**6**) *via* a 'metabolic grid'[9] of isoflavones, isoflavanones, and isoflavanols.‡ The further elaboration in the isoflavonoid B-ring to produce the 2′,4′,5′-substitution pattern in maackiain from the 4′-methoxy-group of (**6**) proceeds by a route which does not involve (**7**) or (**8**).

The two pterocarpans produced by red clover both have the 6a*R*,11a*R*-configuration,[10] and so the enzymic reduction processes leading from (**7**) to (**8**) and (**10**) are presumably stereospecific. If only one enantiomer of (**8**) is involved in the biosynthesis of (**1**), and epimerisation does not occur, the incorporation figures for this isoflavanone may be

† Although feeding conditions during these initial stages were abnormal for the plant, uptake of precursor and production of the pterocarpans were not noticeably different from those observed in experiments carried out under 'normal' conditions.

‡ During preparation of this paper, a communication (M. Cornia and L. Merlini, *J.C.S. Chem. Comm.*, 1975, 428) has appeared in which a chemical analogy for pterocarpan biosynthesis by oxidation of a 2′-hydroxyisoflavan has been reported. The possibility of this oxidation occurring *in vivo* is not excluded by the above results. However, the route proposed here would represent a more direct pathway from isoflavones–isoflavanones to pterocarpans.

corrected accordingly. Of the relatively few naturally-occurring isoflavanones known however, only two, (−)-4′,7-dihydroxyisoflavanone[11] and (3*R*)-sophorol (**11**)[12] have been isolated in optically active form. (3*R*)-Sophorol may well occupy an important position in the biosynthetic pathway to (6a*R*,11a*R*)-maackiain.

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