Stereochemistry of Isoflavone Reduction during the Biosynthesis of (+)- and (−)-Pterocarpans: ^{2}H N.M.R. Studies on the Biosynthesis of (+)-Pisatin and (−)-Medicarpin

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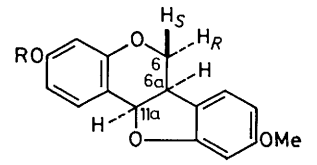
^{2}H N.m.r. spectroscopy has indicated the overall *Z* addition of hydrogen to the double bond of formononetin (**3**) during the biosynthesis of (+)-(6a*R*,11a*R*)-pisatin (**6**), in contrast with an *E* addition during production of (−)-(6a*R*,11a*R*)-medicarpin (**1**).

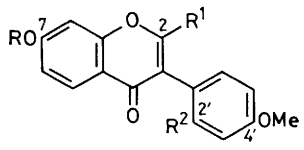
Pterocarpan phytoalexins are synthesized by a wide range of leguminous plants challenged by fungi, viruses, or a variety of abiotic materials.[1] Feeding experiments with radiolabelled precursors in abiotically induced plants[2,3] have demonstrated that (−)-(6a*R*,11a*R*)-medicarpin (**1**)† is biosynthesized from the isoflavone formononetin (**3**) *via* 2′,7-dihydroxy-4′-methoxyisoflavone (**5**) followed by a stereospecific reduction sequence involving the corresponding isoflavone and most likely the isoflavanol. In $CuCl_2$-treated fenugreek (*Trigonella foenum-graecum*) seedlings an overall *E* addition of hydrogen to [2-^{2}H]-(**5**) was observed by the use of ^{2}H n.m.r. spectroscopy.[4] To investigate further this reduction process, and to explore the potential of ^{2}H n.m.r. spectroscopy in plant biosynthetic studies, [2-^{2}H]formononetin (**4**) has been synthesized and tested as a pterocarpan precursor. Its mode of incorporation into (−)-(6a*R*,11a*R*)-medicarpin (**1**) in *T. foenum-graecum* and into the 6a-hydroxypterocarpan of the enantiomeric series (+)-(6a*R*,11a*R*)-pisatin (**6**)† in the garden pea (*Pisum sativum*) has been established, and the results compared.

4-Day-old pea seedlings (from 560 g dry seeds), deprived of endosperm tissue, were treated with aqueous $CuCl_2$ (3×10^{-3} M) *via* the roots for 12 h. The $CuCl_2$ solution was then replaced with a solution of the Na salts of [2-^{2}H]formononetin (96% ^{2}H by ^{1}H n.m.r.; 140 mg) and [*Me*-^{14}C]formononetin (0.45 mg; 2.4×10^6 d.p.m.). Extraction of the plant tissue after a 36 h feeding period yielded (+)-pisatin (40 mg) of specific activity 3.40×10^5 d.p.m./mmol, corresponding to a dilution value of 13.2, and thus 7.6% ^{2}H.

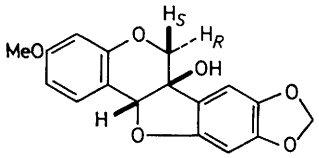
The ^{1}H n.m.r. spectra of pterocarpans have been well studied[7] and analysis of coupling constants has established



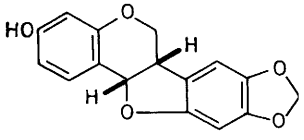
(1) R = H
(2) R = COMe



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



(6)



(7)

the (−)-pterocarpan solution conformation as in Figure 1(A); this conformation has been shown to occur in the solid state also by *X*-ray crystallography.[8] The alternative conformation (B) does not accommodate the n.m.r. data.[7] W-Coupling of 11a-H with 6-H_R (0.6 Hz) is clearly visible in 250 MHz spectra. 6a-Hydroxypterocarpans may be expected to adopt a similar conformation in solution, but no evidence for this has been presented. Substitution of 6a-H by OH simplifies the heterocyclic portion of the ^{1}H n.m.r. spectrum, and W-coupling (0.7 Hz) between 11a-H and the lower-field 6-H doublet at δ 4.00 is again visible in the spectrum of pisatin. It is likely then that the conformation of pisatin in solution is analogous to that of medicarpin [*i.e.* (C) rather than (D)]. Further, lanthanide-induced shift experiments with Eu(fod)$_3$ [tris-

† Pterocarpans having a large negative $[\alpha]_D$ are assigned the (6a*R*,11a*R*) configuration;[5] (—)-6a-hydroxypterocarpans are regarded as having the same absolute configuration,[6] but the priority rules give a (6a*S*,11a*S*) nomenclature.

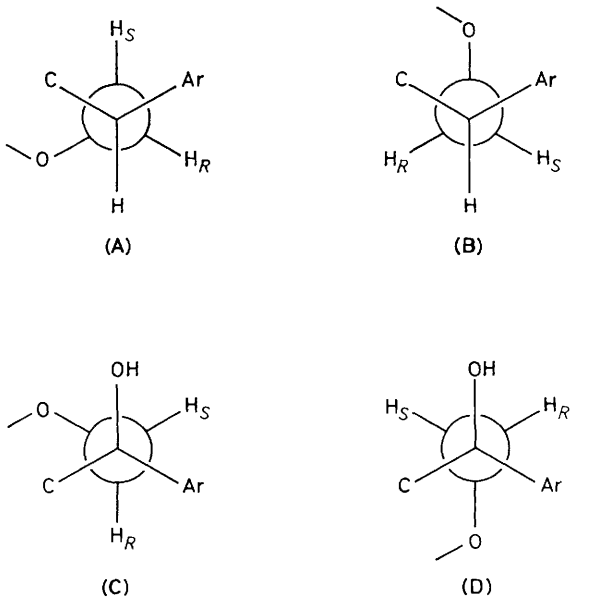


Figure 1. Newman projections along 6a–6 bond for possible conformations of (—)-medicarpin (A and B) and (+)-pisatin (C and D).

(6,6,7,7,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato)europium] show that the low-field 6-H of pisatin is shifted more than the higher-field one, again indicating conformation (C) rather than (D), since in the latter case, equivalent shifts might be expected. Europium-induced shift values (2.1 and 1.8 p.p.m.) were somewhat lower than predicted and suggest that the pisatin–Eu(fod)$_3$ equilibrium lies on the side of the non-complexed species.

The ^{2}H n.m.r. spectrum‡ for the biosynthetically derived (+)-pisatin showed one signal only, at 4.03 p.p.m. [corresponding to 6-H$_R$; δ (6-H$_S$) = 4.18 p.p.m.]. Thus, the elements of water have been added to the double bond of formononetin in an overall *Z* manner. Pisatin is biosynthetically derived by 6a-hydroxylation of maackiain (**7**) followed by methylation,[9] maackiain arising from formononetin by way of 3′,7-dihydroxy-4′-methoxy-, 7-hydroxy-3′,4′-methylenedioxy-, and 2′,7-dihydroxy-4′,5′-methylenedioxy-isoflavones, and subsequent reduction.[10] Recent studies[11] have further established that (+)-pisatin is produced by hydroxylation of (+)-(6a*S*,-11a*S*)-maackiain (**7**) with retention of configuration at C-6a. The possible involvement of pterocarp-6-ene or pterocarp-6a-ene derivatives between pterocarpans and 6a-hydroxypterocarpans and a dehydrogenation–hydration mechanism can be excluded.[11] Hence, since (+)-maackiain is an intermediate between formononetin and (+)-pisatin, it may be inferred that the biosynthesis of (+)-maackiain must involve an overall *Z* addition of hydrogen to the double bond. This contrasts with the overall *E* addition of hydrogen to the double bond of the isoflavone (**5**) observed during the biosynthesis of (−)-medicarpin,[4] and confirmed by a similar experiment using [2-^{2}H]formononetin. Again, the ^{2}H n.m.r. spectrum showed only one signal corresponding to 6-H$_R$.

This means that the stereochemistry of the reduction process leading to (+)-maackiain and (−)-medicarpin is different, and not merely similar reduction but from the opposite face of the isoflavone. If this is a general feature of pterocarpan biosynthesis, *i.e.* that (−)-pterocarpans are produced by *E* reduction and (+)-pterocarpans by *Z* reduction, it could be reflected in the preponderance of (−)-pterocarpans over (+)-isomers in nature.[12] A small number of plants though are known to accumulate both (+)- and (−)-isomers. Except for (+)-pisatin, all the pterocarpans *acting as phytoalexins* so far reported appear to have negative optical rotations. It is an unusual feature of *P. sativum* that a minor phytoalexin produced simultaneously with (+)-pisatin is (−)-maackiain.[11,13] Unfortunately, the very small amounts of (−)-maackiain produced do not permit comparison of its biosynthesis with that of (+)-pisatin by the present method.

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‡ Proton-noise-decoupled ^{2}H n.m.r. spectra were run at 38.4 MHz in $CHCl_3$ soln. using natural abundance $CDCl_3$ as internal standard.

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