Stereochemistry of Isoflavone Reduction during Pterocarpan Biosynthesis: an Investigation using Deuterium Nuclear Magnetic Resonance Spectroscopy

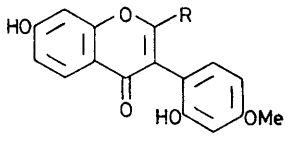
By PAUL M. DEWICK* and DAVID WARD

(*Department of Pharmacy, University of Nottingham, University Park, Nottingham* NG7 2RD)

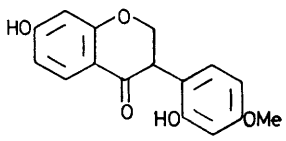
Summary ^{2}H N.m.r. spectroscopy has been employed to establish that in fenugreek seedlings, (6a*R*,11a*R*)-demethylhomopterocarpin is synthesised from 2′,7-dihydroxy-4′-methoxyisoflavone *via* an overall *trans* addition of hydrogen to the double bond.

FEEDING experiments[1,2] using ^{14}C-labelled compounds have demonstrated that 2′,7-dihydroxy-4′-methoxy-isoflavone (**1**) and -isoflavanone (**3**) are excellent biosynthetic precursors of the pterocarpan phytoalexin (6a*R*,11a*R*)-demethylhomopterocarpin (**4**) in $CuCl_2$-treated seedings of red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*). The biosynthetic pathway to (**4**) most probably proceeds *via* reduction of (**1**) to (**3**), further reduction to the isoflavanol (**6**), then cyclisation to the pterocarpan. An intermediate carbonium ion (**7**), or its mesomeric counterpart, has been postulated.[2]

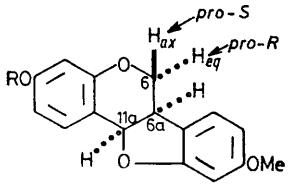
The stereochemical features of the reduction sequence from (**1**) to (**4**) have been investigated in seedlings of fenugreek (*Trigonella foenum-graecum*), which accumulate significant amounts of this pterocarpan on treatment with $CuCl_2$ and upon irradiation with u.v. light. 2′,7-Dihydroxy-4′-methoxy[2-^{2}H]isoflavone (**2**) [386 mg; ^{2}H enrichment 96% from mass spectroscopy (m.s.) data, 97%



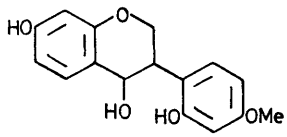
(1) R = H
(2) R = ^{2}H



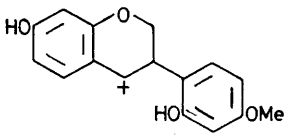
(3)



(4) R = H
(5) R = COMe



(6)



(7)

from ^{1}H n.m.r.], as its Na-salt, was administered in aqueous buffer to the roots of fenugreek seedlings treated with $CuCl_2$ and irradiated with u.v. light (4 day old, from 160 g seeds) over 17 h. Work up of the plant material yielded (**4**), which was purified as its acetate (**5**) (45 mg; ^{2}H enrichment 60% from m.s.). The location on the ^{2}H-label was established by both ^{2}H and ^{1}H n.m.r. spectroscopy.

The ^{1}H n.m.r. signals arising from the heterocyclic ring hydrogens of pterocarpans have been extensively studied and unambiguously assigned.[3] In particular, the 6-*pro-R* (6-equatorial) hydrogen resonates at a lower (*ca.* 0·5—0·6 p.p.m.) field than the 6-*pro-S* (6-axial) hydrogen. A sample of (±)-[6-^{2}H]-(**5**) (48% ^{2}H enrichment at each 6-H), prepared from (**2**) *via* $NaBH_4$ reduction to the pterocarpan, showed two peaks in its ^{2}H n.m.r. spectrum† at 3·93 and 3·44 p.p.m, together with the natural abundance [^{2}H]-benzene peak at 7·27 p.p.m. Since ^{2}H and ^{1}H chemical shifts are identical,[5] these two peaks can be assigned to ^{2}H in the 6-*pro-R* and 6-*pro-S* positions, respectively. However, the sample of (**5**) derived from the [^{2}H]isoflavone feeding experiment showed only a single signal at 3·92 p.p.m. in its ^{2}H spectrum, indicating that the ^{2}H label had been incorporated exclusively into the 6-*pro-R* position. By a comparison of peak heights in the spectra of the synthetic and biosynthetic samples in relation to the natural abundance [^{2}H]benzene signal, the ^{2}H enrichment of the biosynthetic pterocarpan was estimated to be 56%. The result was confirmed by the ^{1}H n.m.r. spectrum, the 6-*pro-R* signal being reduced in intensity (58% ^{2}H enrichment by comparison with the 11a proton integral). Thus, in fenugreek, (6a*R*,11a*R*)-demethylhomopterocarpin is synthesised from 2′,7-dihydroxy-4′-methoxyisoflavone *via* an overall *trans* addition of hydrogen to the double bond, hydrogen being added to C-2 from the *si* face.

To date, there are very few reports[6] of the application of ^{2}H n.m.r. spectroscopy in biosynthetic studies, and these have been concerned with microbial systems. Here, we have illustrated the potential of the technique in plant systems. In the present studies, the dilution (1·6) was sufficiently small that meaningful results could be obtained by analysis of the ^{1}H n.m.r. spectrum. However, our studies have indicated that with dilution values of 10, or even higher, one may obtain quite satisfactory ^{2}H n.m.r. spectra. Under such conditions, analysis of the ^{1}H n.m.r. spectra to measure ^{2}H enrichment becomes less reliable.

We thank the S.R.C. for financial support, and Professor L. Crombie, Chemistry Department, for n.m.r. facilities.

(*Received, 7th March* 1977; *Com.* 211.)

† Spectra were measured using benzene solutions to take advantage of the upfield solvent-induced shift (see ref. 4) for the methoxy signal. Pulsed Fourier transform n.m.r. spectra (15·35 MHz, JEOL, PFT-100/Nicolet 1080A, proton-noise decoupled) were obtained from solutions in 10 mm coaxial tubes using C_6F_6 as external lock, and natural abundance [^{2}H]benzene as internal standard. Added $CDCl_3$ proved unsuitable as an internal standard because of its solvent-induced shift in benzene.

[1] P. M. Dewick, *J.C.S. Chem. Comm.*, 1975, 656; *Phytochemistry*, 1977, **16**, 93.
[2] P. M. Dewick and M. Martin, *J.C.S. Chem. Comm.*, 1976, 637.
[3] K. G. R. Pachler and W. G. E. Underwood, *Tetrahedron*, 1967, **23**, 1817.
[4] A. Pelter and P. I. Amenechi, *J. Chem. Soc.* (*C*), 1969, 887.
[5] P. Diehl, in 'Nuclear Magnetic Resonance Spectroscopy of Nuclei other than Protons,' eds. T. Axenrod and G. A. Webb, Wiley-Interscience, New York, 1974, p. 275.
[6] B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 123; Y. Sato, T. Oda, and H. Saito, *Tetrahedron Letters*, 1976, 2695.