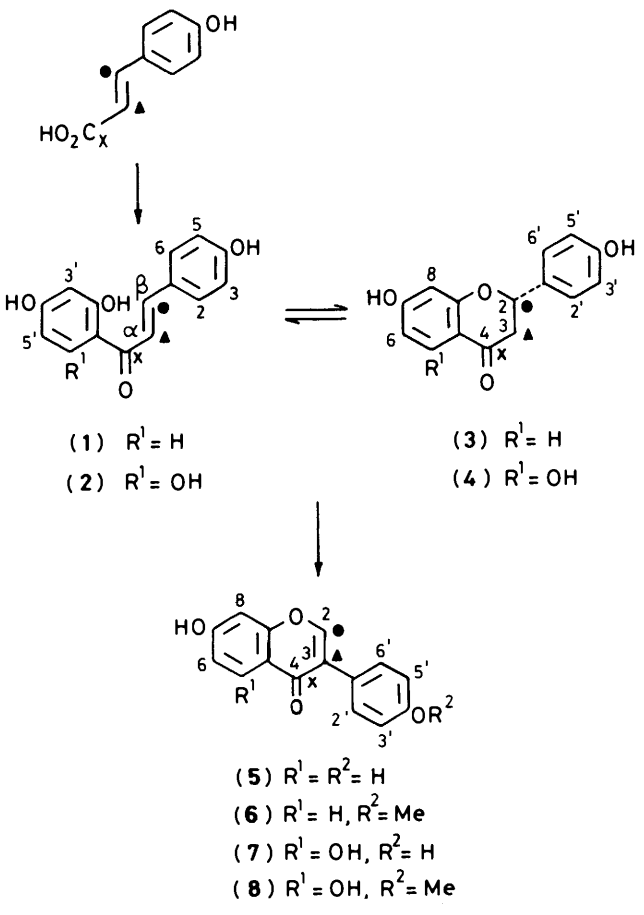
Isoflavonoid Biosynthesis: Concerning the Aryl Migration

Hakim A. M. Al-Ani and Paul M. Dewick *
Department of Pharmacy, University of Nottingham, Nottingham NG7 2RD

Feeding experiments with ^{13}C- or ^{2}H-labelled precursors in $CuCl_2$-treated red clover (*Trifolium pratense*) seedlings have demonstrated that the isoflavone formononetin (**6**) and the pterocarpan phytoalexins medicarpin (**10**) and maackiain (**11**) are biosynthesized from 2',4,4'-trihydroxychalcone by a rearrangement process involving an intramolecular migration of the cinnamate-derived aromatic ring. In all three compounds, this is accompanied by retention of the chalcone's β-hydrogen but loss of the α-hydrogen. During the formation of maackiain from formononetin, an NIH shift of ^{2}H as a result of aromatic hydroxylation *ortho* to the methoxy group was observed. Experiments with 7-hydroxy-4'-methoxy[2-$^{2}H_2$]isoflavanone indicate that this compound may be converted into medicarpin without loss of ^{2}H, thus confirming the existence of a metabolic grid of isoflavones and isoflavanones. The results are best explained in terms of an oxidative process in which a chalcone is converted into an isoflavone as the first-formed isoflavonoid derivative.

Isoflavonoids are formed in nature from flavonoid C_6-C_3-C_6 precursors produced by condensation of acetate-malonate units onto a cinnamic acid starter molecule.[1] During the biosynthesis of isoflavonoids however, the phenylalanine/cinnamic acid derived aromatic ring migrates to the adjacent carbon of the C_3 unit (Scheme 1). By the feeding of specifically ^{14}C-labelled

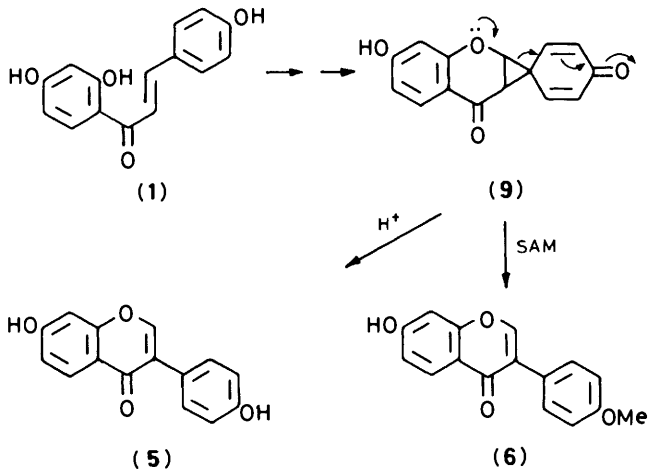


Scheme 1.

phenylalanine, cinnamic acid, or chalcones to various plant systems, followed by chemical degradation of the labelled isoflavonoid produced, this unusual aryl migration has been formally demonstrated to occur during the biosynthesis of three of the several classes of isoflavonoids, namely isoflavones,[2] coumestans[3] and rotenoids.[4] There is now considerable evidence to suggest that the modified isoflavonoid derivatives, *e.g.* coumestans, rotenoids, pterocarpans, isoflavans, are all derived from primary rearrangement products, probably simple isoflavones such as daidzein (**5**), formononetin (**6**), genistein (**7**) or biochanin A (**8**).[5] These four isoflavones are produced from two chalcone/flavanone precursors only, (**1**)/(**2**) or (**3**)/(**4**), precursors having other substitution patterns in the migrating aryl being unacceptable for isoflavonoid biosynthesis. Even the appropriate 4-methoxy chalcones/flavanones are transformed into formononetin or biochanin A by processes involving removal of the original methyl group.[6] Whether a chalcone or a flavanone is the more immediate precursor of isoflavonoids is not known with any certainty. There is some evidence from competitive feeding experiments[7] that chalcones are the immediate precursors, but the ready enzymic isomerization of chalcones and flavanones leaves the problem still unresolved.

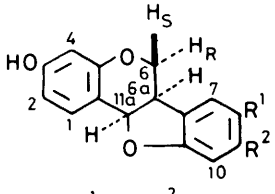
The mechanism of the aryl migration is also completely unknown. Over the years, many hypotheses have been proposed, some based on synthetic approaches to isoflavones from chalcone/flavonoid intermediates,[8] others being purely speculative. As yet, none of these fits in with the biosynthetic evidence available, and perhaps the true answer must await the isolation of the enzyme catalysing this crucial migration step. The enzyme(s) have a very limited distribition in nature, isoflavonoids being essentially restricted to the Leguminosae.[9] The biosynthetic evidence is that the reaction appears to be oxidative in nature, and the probable site of oxidation is the 4-hydroxy of the migrating aryl. 4'-Methoxyisoflavones seem to acquire their methyl group by a process other than methylation of the 4'-hydroxyisoflavone. Thus, 2',4,4'-trihydroxychalcone (**1**) is a good precursor of formononetin (**6**) whereas daidzein (**5**) is poorly utilized.[10,11] One mechanism proposed, including all of these features, is that of Pelter, Bradshaw, and Warren,[12] who propose a spirodienone intermediate (**9**) (Scheme 2), formed by phenolic oxidation of a chalcone, followed by proton- or S-adenosyl methionine-catalysed decomposition leading to 4'-hydroxy- or 4'-methoxy-isoflavones respectively. Whilst chemically reasonable, this mechanism is unfortunately much less attractive in terms of enzymic processes.

It is almost 25 years since the aryl migration in the biosynthesis of isoflavones was demonstrated by ^{14}C labelling studies.[2] Any analogy with the acid-catalysed rearrangement of chalcone epoxides to isoflavones was ruled out since there an aroyl migration was observed.[13] Whilst the aryl migration is not



Scheme 2.

doubted, it seemed appropriate to use modern ^{13}C n.m.r. methods to confirm the earlier data. Acylation of resorcinol with [1,2-$^{13}C_2$]acetyl chloride (*ca.* 90% at each C) gave [1,2-$^{13}C_2$]resacetophenone which on base condensation with 4-hydroxybenzaldehyde gave 2′,4,4′-trihydroxy[*carbonyl*,α-$^{13}C_2$]chalcone (**12**). This chalcone (1.2 g) together with tracer amounts (6.25 mg) of the trihydroxy[*carbonyl*-^{14}C]chalcone was fed to 4-day-old red clover (*Trifolium pratense*) seedlings (*ex.* 310 g seeds), previously treated with aqueous $CuCl_2$ for 8 h. Under these induction conditions, red clover synthesizes the pterocarpans (6a*R*,11a*R*)-medicarpin (**10**) and (6a*R*,11a*R*)-maackiain (**11**) as phytoalexins by a biosynthetic pathway

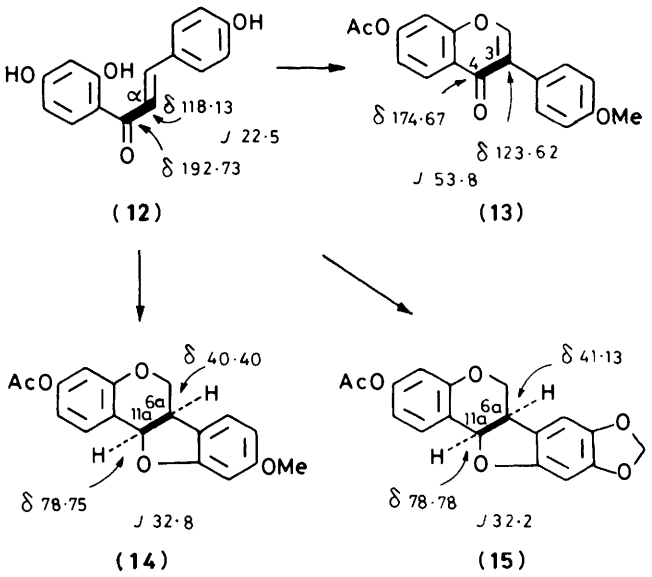


(**10**) R^1 = H, R^2 = OMe

(**11**) R^1R^2 = OCH_2O

involving trihydroxychalcone (**1**) and formononetin (**6**).[11,14,15] Formononetin itself is produced constitutively in red clover seedlings as the major phenol. After a feeding period of 16 h, medicarpin (58 mg), maackiain (60 mg), and formononetin (140 mg) were isolated from the plant tissue, converted into their acetates, and purified to constant specific activity by t.l.c. and recrystallizations. Dilution values (^{14}C) for formononetin, medicarpin and maackiain were 34.3, 19.5, and 27.4 respectively, corresponding to specific incorporations/enrichments of 2.9, 5.1, and 3.6% respectively. The ^{13}C n.m.r. spectra of these materials in each case showed only two signals that were flanked by ^{13}C–^{13}C satellites, C-3 and C-4 in the case of formononetin acetate (**13**), and C-6a and C-11a in the case of the pterocarpan acetates (**14**) and (**15**) (Scheme 3). The intensities of the satellites were in excellent agreement with the ^{14}C specific incorporations, after correcting for single-labelled species in the chalcone fed. The retention of intact ^{13}C–^{13}C linkages from the chalcone to the three isoflavonoids shows that the C_3 chain of the chalcone is not rearranged and thus confirms that an aryl migration has occurred in the biosynthesis.

The intramolecular nature of the aryl migration is confirmed for the first time by the results from a feeding experiment with



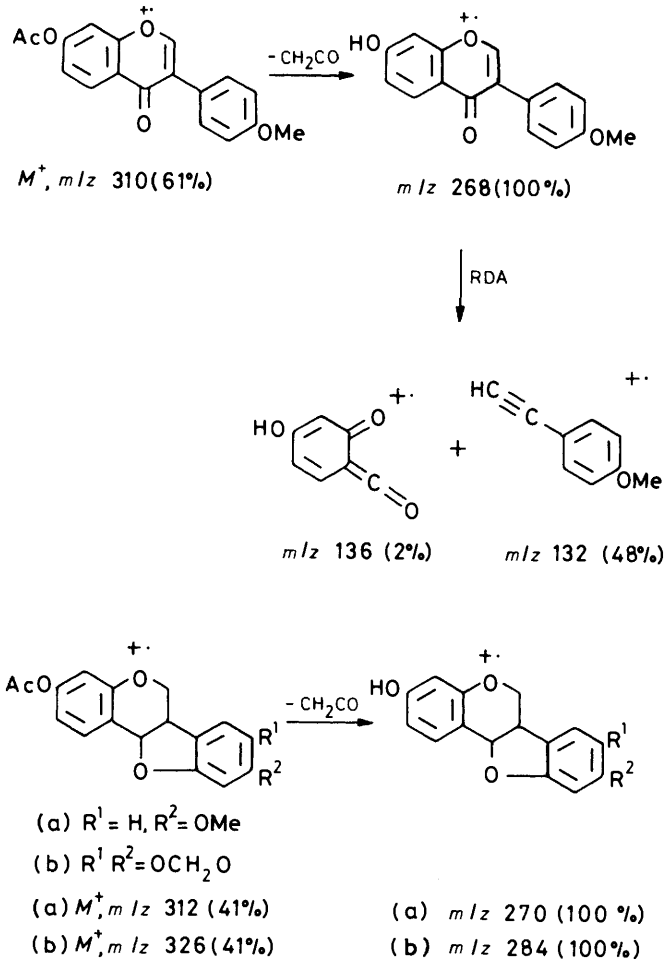
Scheme 3. *Solvents:* (**12**), (**14**), and (**15**) $(CD_3)_2CO$; (**13**) $(CD_3)_2SO$

2′,4,4′-trihydroxy[β,3,5-$^2H_3$]chalcone (**21**), synthesized from resacetophenone and 4-hydroxy[*formyl*,3,5-$^2H_3$]benzaldehyde. 4-Benzyloxy[*formyl*-^{2}H]benzaldehyde, prepared according to the general method of Bennett *et al.*[16] *via* α-4-benzyloxyphenyl-α-morpholinoacetonitrile, was debenzylated, and then deuteriated in the 3,5-positions by repeated exchange with ^2H_2O under alkaline conditions[17] to yield the trideuteriated aldehyde. ^1H N.m.r. spectroscopy indicated the product had 93, 93, and 95% ^2H at positions 3,5, and formyl respectively. After condensation with resacetophenone, the ^2H content of the resultant chalcone was unchanged.

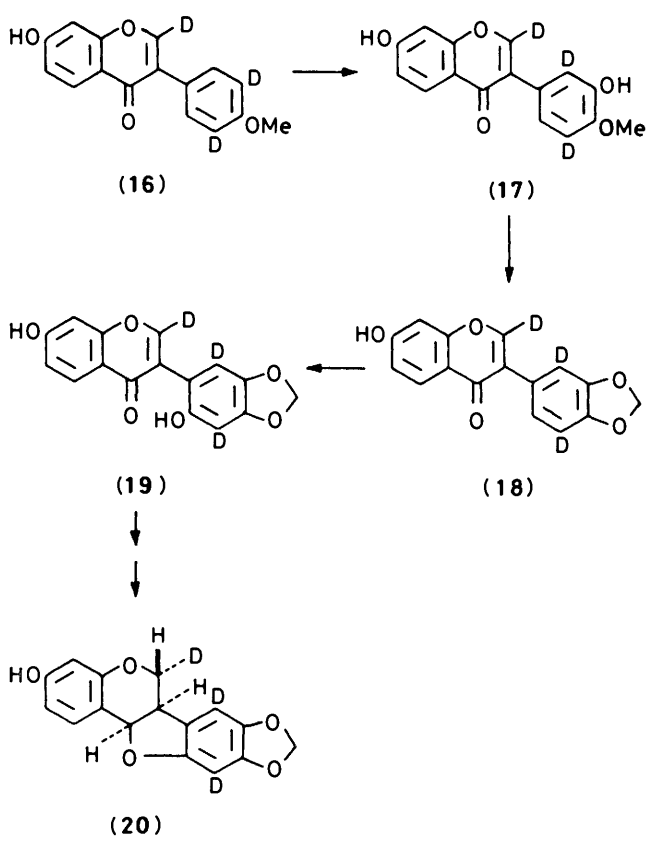
This chalcone (152 mg), together with a tracer amount (0.81 mg) of [^{14}C]chalcone, was fed to $CuCl_2$-induced red clover seedlings (*ex.* 30 g seeds) to yield formononetin (9.5 mg), medicarpin (3.0 mg) and maackiain (6.0 mg). These compounds were again rigorously purified as their acetates, and ^{14}C counting indicated isotopic dilution values of 17, 21, and 23 respectively. Mass spectral analysis of these products (Table 1) showed the presence of [$^2H_3$]-labelled molecular ions for formononetin and medicarpin, the calculated proportions being in good agreement with the ^{14}C specific incorporations. The production of [$^2H_3$]isoflavonoid from [$^2H_3$]chalcone, labelled on the C_3 chain and migrating aryl, demonstrates that the rearrangement must, therefore, be intramolecular. In both of these spectra, the $M - 42$ (loss of acetyl) peak similarly showed the presence of three deuterium labels. With formononetin acetate, a significant fragment is *m*/*z* 132 (Scheme 4) resulting from RDA fission. This peak again indicated the presence of three deuterium labels (*m*/*z* 135) in complete accord with the labelling pattern expected in the product, in particular the retention of the chalcone β-hydrogen as 2-H of the isoflavone.[18]

Table 1. Incorporation of 2′,4,4′-trihydroxy[β,3,5-$^2H_3$]chalcone

Compound isolated	Dilution	% ^2H (^{14}C)	Mass spectral analysis (M^+, %) $^2H_0$	$^2H_1$	$^2H_2$	$^2H_3$
Formononetin	17	5.9	92.7	0	0.8	6.4
Medicarpin	21	4.8	94.3	0.6	1.1	4.1
Maackiain	23	4.3	95.5	0	2.4	2.1



Scheme 4. Major mass spectral fragments for formononetin, medicarpin and maackiain acetates

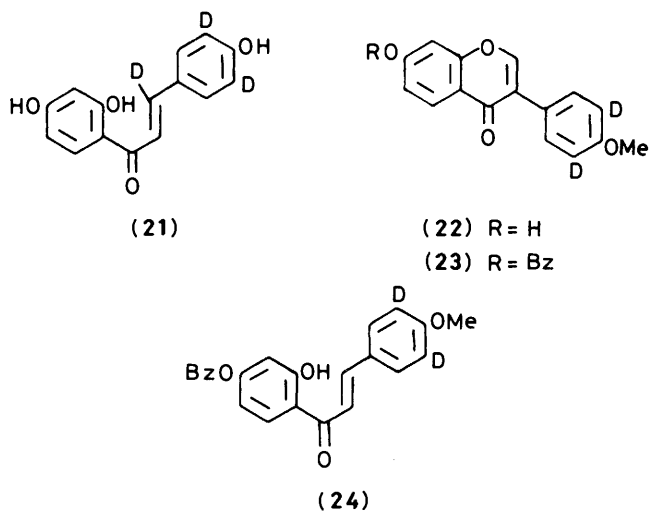


Scheme 5.

Unfortunately, EI mass spectral fragmentation of pterocarpans gives relatively weak ions and at this level of deuterium content, little additional information about the labelling pattern in medicarpin.

The maackiain acetate mass spectrum showed that approximately half of the ^{2}H-labelled molecular ions contained two deuterons, and the other half contained three deuterons, a result also reflected in the $M - 42$ fragment. This interesting result undoubtedly arises through an NIH shift of deuterium from C-8 of the pterocarpan to presumably C-7 as a consequence of the additional hydroxylation during formation of the methylenedioxy group. The biosynthetic pathway to maackiain has been demonstrated[15] to involve hydroxylation of formononetin to 3′-hydroxyformonetin (**17**) (Scheme 5), formation of the methylenedioxy group *via* pseudobaptigenin (**18**), then 2′-hydroxylation, stepwise reduction, and cyclization. From empirical rules relating to the NIH shift,[19] one may expect migration of ^{2}H-label on *ortho* hydroxylation of a 4-methoxy system, but not on *ortho* hydroxylation of a 4-hydroxylated ring. Thus, the observed NIH shift during the biosynthesis of maackiain from 2′,4,4′-trihydroxy[β,3,5-$^2H_3$]-chalcone (**21**) {*via* presumably, [2,3′,5′-$^2H_3$]formononetin (**16**)} confirms, in part, the pathway elucidated from ^{14}C incorporation studies, *i.e.* hydroxylation of a 4-methoxy- rather than a 4-hydroxy-isoflavone and thus formononetin→3′-hydroxyformononetin (Scheme 5).

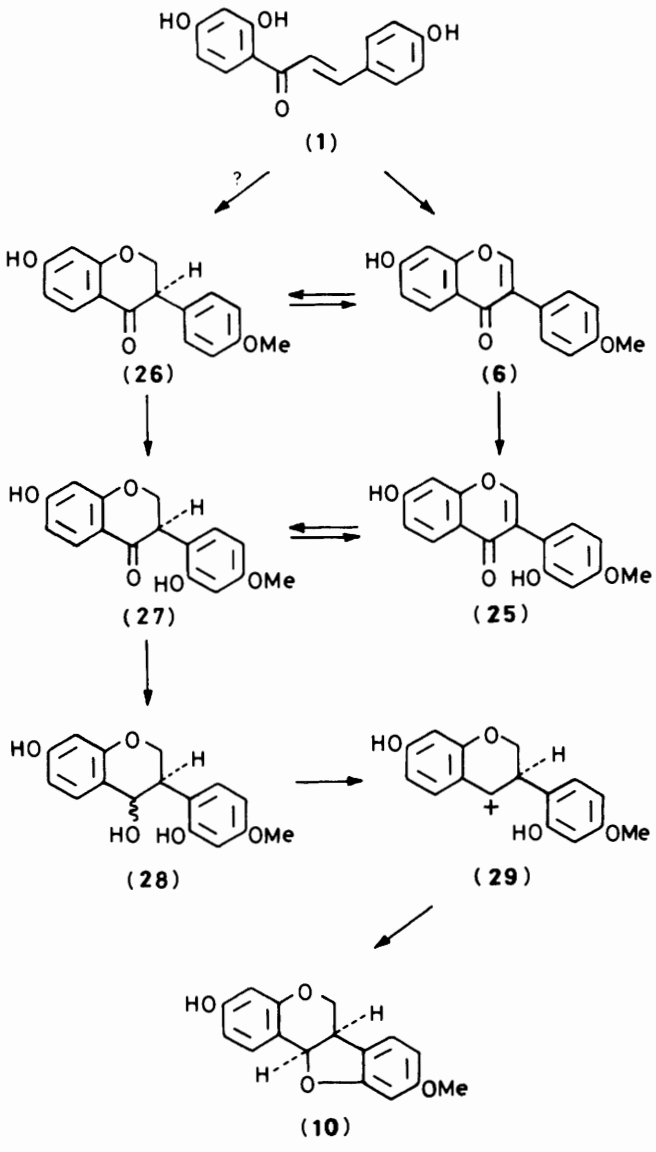
This NIH shift was studied further by feeding [3′,5′-$^2H_2$]-formononetin (**22**) to red clover seedlings. 4-Hydroxy[3,5-$^2H_2$]benzaldehyde (*cf.* above), was methylated, then base condensation with 4′-*O*-benzylresacetophenone yielded 4′-benzyloxy-2′-hydroxy-4-methoxy[3,5-$^2H_2$]chalcone (**24**). This chalcone was converted into 7-benzyloxy-4′-methoxy[3′,5′-$^2H_2$]isoflavone (**23**) *via* $Tl(NO_3)_3$ oxidation[20] and the benzyl



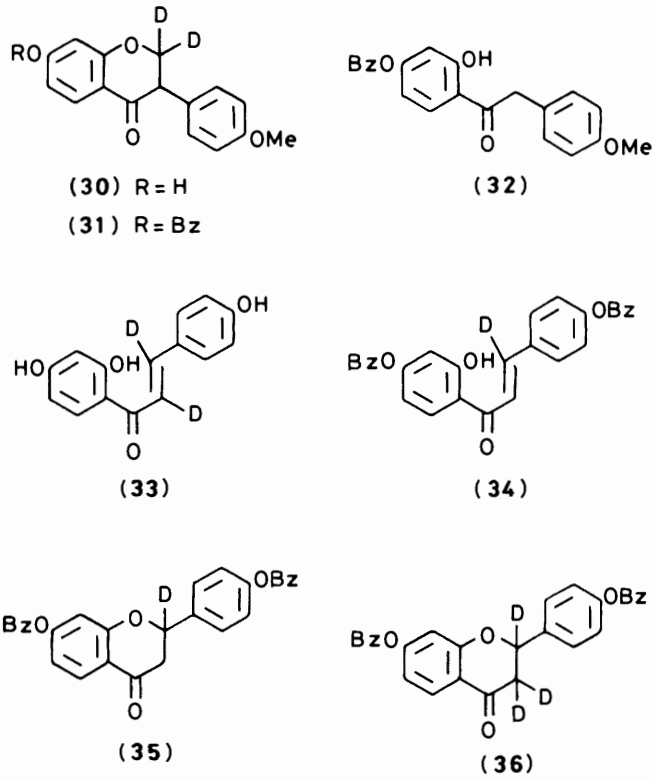
group was removed by $Pd(OH)_2$–C/cyclohexene catalytic transfer hydrogenation[21] to give [3′,5′-$^2H_2$]formononetin containing approximately 95% ^{2}H at each position. The labelled isoflavone (650 mg), together with [*methyl*-^{14}C]formononetin (3.1 mg) was fed as before to $CuCl_2$-induced

red clover seedlings (*ex.* 310 g seeds) giving maackiain (62 mg), purified as its acetate. Its isotopic dilution of 47.2 corresponded, therefore, to a specific incorporation of 2.1%. ^2H N.m.r. of this maackiain acetate showed two peaks at 7.08 and 6.54 p.p.m., corresponding therefore to ^2H at 7-H and 10-H, in agreement with an NIH shift as predicted (see Experimental section for ^1H n.m.r.). The intensities of these two signals were in the ratio of approximately 1:2, correlating very well with the mass spectral data above, 2.1:4.5≡1:2.1 (Table 1).

During studies of the biosynthesis of medicarpin in $CuCl_2$-treated lucerne (*Medicago sativa*) seedlings,[22] we had noted the excellent incorporation of ^{14}C-labelled formononetin, 2′,7-dihydroxy-4′-methoxyisoflavone (**25**), (±)-7-hydroxy-4′-methoxyisoflavanone (**26**) and (±)-2′,7-dihydroxy-4′-methoxyisoflavanone (**27**) into the pterocarpan. Incorporations of 2.1–6.6% were measured. If the pathway to medicarpin proceeds *via* 2′,7-dihydroxy-4′-methoxyisoflavanol (**28**), by analogy with synthetic routes to pterocarpans,[23] these high incorporation figures offer substantial evidence that the transformation of formononetin to medicarpin may be accomplished *via* a metabolic grid of isoflavones and isoflavanones, and perhaps also isoflavanols (Scheme 6). In this case, there may be no unique pathway from (**6**) to (**10**). However, because isoflavanones may be oxidized to isoflavones *in vivo*,[18] the incorporation of 7-hydroxy-4′-methoxyisoflavanone (**26**) *via* formononetin (**6**) could not be excluded. Formononetin, (**25**), and (**27**) have also been shown to be excellent precursors of medicarpin in red clover, with incorporations of 0.62—12.8%,[14] and a metabolic grid could also exist in this plant. In further experiments (Table 2), high incorporations of [*methyl*-^{14}C]-labelled formononetin and (±)-7-hydroxy-4′-methoxyisoflavanone into both pterocarpan phytoalexins were observed in tracer studies (*ca.* 0.4 mg fed to seedlings from 2 g seeds). However, good incorporation of the isoflavanone into formononetin was also noted. To establish beyond doubt the role of metabolic grids in isoflavonoid biosynthesis, a further feeding experiment using (±)-7-hydroxy-4′-methoxy[2-$^2H_2$]-isoflavanone (**30**) was performed.



Scheme 6.



Synthesis of this compound was achieved by prolonged stirring of the deoxybenzoin 4-methoxybenzyl 4-benzyloxy-2-hydroxyphenyl ketone (**32**) with $C^2H_2I_2$ (99% ^2H) in dry acetone–K_2CO_3.[24] The benzyl ether (**31**) was converted into the phenol by catalytic transfer hydrogenation. This isoflavanone (151 mg) together with tracer amounts (0.61 mg) of [*methyl*-^{14}C]isoflavanone was then fed to $CuCl_2$-treated red clover seedlings (*ex.* 30 g seeds) to give, after work-up and purification, formononetin acetate (20 mg), medicarpin acetate (4.6 mg), and maackiain acetate (3.5 mg). Isotopic dilutions were 34.6, 19.6, and 113 respectively, corresponding to specific incorporations 2.9, 5.1, and 0.9% respectively. Mass spectral analysis of these compounds (Table 3) showed the presence of a molecular ion for [$^2H_1$]formononetin acetate at a level in reasonable agreement with the specific incorporation figure, together with fragments at *m/z* 269 and 133, corresponding also to monodeuteriated species (Scheme 4). Medicarpin acetate showed molecular ions corresponding mainly to [$^2H_2$]medicarpin acetate and much lower levels of [$^2H_1$]medicarpin acetate, the combined amounts approximating to the specific

Table 2. Incorporation of [*methyl*-^{14}C]-labelled formononetin and (±)-7-hydroxy-4′-methoxyisoflavanone *

Compound fed	Formononetin		Medicarpin		Maackiain	
	Dilution	% Incorpn.	Dilution	% Incorpn.	Dilution	% Incorpn.
Formononetin	5.6	61.1	46	1.84	83	0.79
(±)-7-Hydroxy-4′-methoxyisoflavanone	411	0.64	132	0.65	283	0.26

* Not corrected for possible utilization of one enantiomer

Table 3. Incorporation of (±)-7-hydroxy-4′-methoxy[2-$^2H_2$]isoflavanone

Compound isolated	Dilution	% ^{2}H (^{14}C)	Mass spectral analysis (M^+,%)		
			$^2H_0$	$^2H_1$	$^2H_2$
Formononetin	34.6	2.9	97.3	2.2	0.5
Medicarpin	19.6	5.1	95.1	0.3	4.6
Maackiain	113	0.9			

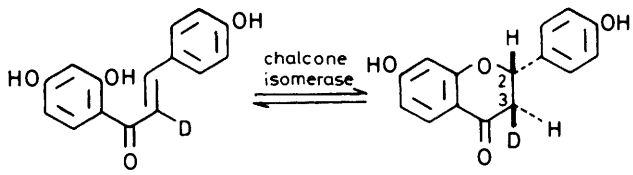
incorporation figure. Dideuteriated species also predominated in the M − acetyl fragment m/z 270. This result shows that 7-hydroxy-4′-methoxyisoflavanone is converted into medicarpin mainly by a route involving no loss of 2-H labels, *i.e.* not *via* formononetin. The proportion of the isoflavanone converted into formononetin and then on to medicarpin is surprisingly low in view of the good conversion into formononetin (Table 2), and the excellent incorporations of formononetin into medicarpin (Table 2). Unfortunately, in this experiment, the incorporation levels into maackiain were too low to enable any accurate level of ^{2}H enrichment in this pterocarpan to be measured by mass spectral methods. Thus, overall the production of [$^2H_2$]medicarpin now proves the existence of a metabolic grid in red clover. Based solely on incorporation data from this and previous experiments,[14] the sequence from formononetin to medicarpin *via* isoflavone (**25**) and isoflavanone (**27**) would seem to be more important than that *via* isoflavanones (**26**) and (**27**).

Whilst the transformation of a chalcone to an isoflavone is an overall oxidative conversion involving loss of two hydrogen atoms, there is as yet no definite proof that an isoflavone is the first-formed product from the aryl migration process. The possibility that the primary product may be an isoflavanone has also been considered[18] but not excluded. With the knowledge that 7-hydroxy-4′-methoxyisoflavanone may be converted into medicarpin without passing through an isoflavone intermediate, there exists the possibility that α-H of a chalcone may be retained if the sequence is chalcone→isoflavanone→pterocarpan. This hydrogen must be lost if isoflavone intermediates are involved. To test this hypothesis, 2′,4,4′-trihydroxy[α,β-$^2H_2$]chalcone (**33**) was incorporated into medicarpin.

4,4′-Dibenzyloxy-2′-hydroxy[β-^{2}H]chalcone (**34**) from base condensation of 4′-benzyloxy-2′-hydroxyacetophenone with 4-benzyloxy[*formyl*-^{2}H]benzaldehyde was converted into the isomeric 4′,7-dibenzyloxy[2-^{2}H]flavanone (**35**) by treatment with aqueous NaOAc.[25] Exchange of the protons at C-3 was accomplished by stirring with basic alumina in ^2H_2O,[18] then the flavanone (**36**) was isomerized back to the [α,β-$^2H_2$]chalcone by ring opening with NaO^{2}H/^2H_2O.[26] Debenzylation with BBr_3 [26] yielded the required 2′,4,4′-trihydroxy[α,β-$^2H_2$]chalcone containing 94% ^{2}H at each position. This chalcone (1.4 g) together with tracer [^{14}C]chalcone (7.9 mg) was fed to induced red clover seedlings (*ex.* 310 g seeds) as previously to give formononetin (148 mg), medicarpin (57 mg) and maackiain (62 mg), isolated as their acetates. Isotopic dilution values (^{14}C) of 32.5, 19.7, and 22.4 respectively corresponded to ^{2}H enrichments of 3.1, 5.1, and 4.4%.

In the ^{2}H n.m.r. spectra for these compounds, only one peak was observed in each case, at δ 8.58 p.p.m. for formononetin acetate (corresponding to 2-H), and at δ 4.43 p.p.m. for medicarpin and maackiain acetates (corresponding to ^{2}H at 6-H-*pro-R* in each compound). These signals represent the retention of β-H from the chalcone into the isoflavone and also into the pterocarpans. In the pterocarpans, the location of deuterium is entirely in accord with previous studies[27] in which [2-^{2}H]-labelled isoflavones served as precursors of medicarpin in *Trigonella foenum-graecum via* an overall *E* addition of hydrogen to the double bond. However, for all three compounds, α-H from the chalcone appeared to be lost.

This loss is most probably accounted for by the involvement of isoflavone intermediates and favours the biosynthetic transformation of a chalcone to an isoflavone as the first-formed isoflavonoid intermediate. Unfortunately, this negative result is less easy to interpret than a positive one, for the chalcone's α-H could be removed by at least two other processes. This hydrogen is susceptible to exchange in an isoflavanone intermediate, although synthetic sequences with [3-^{3}H]isoflavanones[28] have indicated exchange losses may not necessarily be large. Alternatively, an aryl migration mechanism involving a flavanone could, in fact, give an isoflavanone but with loss of the chalcone's α-hydrogen. It is known that the α-hydrogen of a chalcone becomes the 3-*pro-R* hydrogen of a (2*S*)-flavanone during the chalcone isomerase conversion[26] (Scheme 7). Consequently, this hydrogen is most



Scheme 7.

likely to be the one eliminated in an *anti* migration process. The results obtained in these studies indicate that the formation of isoflavonoids from chalcones is the result of an intramolecular 1,2-aryl migration in which the chalcone's β-hydrogen is retained, and its α-hydrogen is lost. Such results are still best explained in terms of an oxidative process in which an isoflavone is the first-formed isoflavonoid.

Experimental

M.p.s were determined on a Buchi 510 apparatus. U.v. spectra were measured in EtOH solution using a Perkin-Elmer 554 spectrometer. ^{1}H N.m.r. spectra were recorded at 60 MHz and 250 MHz using Varian EM 360 and Bruker WM 250 instruments respectively. ^{13}C and ^{2}H N.m.r. spectra were obtained using the latter instrument at 62.89 and 38.40 MHz respectively. All chemical shifts are relative to tetramethylsilane standard. Mass spectra were obtained on an A.E.I. MS 902 instrument at 200 °C and 70 eV. T.l.c. was carried out using 0.5 mm or 1.0 mm layers of silica gel (Merck Kieselgel GF_{254}) on 20 × 20 cm or 20 × 40 cm glass plates. T.l.c. zones were eluted with AnalaR acetone. Radioactive samples were counted in dioxane-based or toluene-based liquid scintillator (B.D.H.)

using a Tracerlab Corumatic 200 l.s. spectrometer. Efficiencies were determined using toluene internal standards. The syntheses of 2′,4,4′-trihydroxy[*carbonyl*-^{14}C]chalcone (0.514 mCi/mM),[29] [*methyl*-^{14}C]formononetin (0.568 mCi/mM)[22] and (±)-7-hydroxy-4′-methoxy[*methyl*-^{14}C]isoflavanone (0.676 mCi/mM)[22] were carried out according to previously published procedures.

Plant Material, Feeding Techniques and Isolation of Metabolites.—Seeds (310 g) of red clover (*Trifolium pratense*) "Essex Cert." (Dickson, Brown, and Tait, Ltd., Altrincham) were sown evenly onto layers of moist filter paper in six trays (46 × 56 cm) containing a 3 cm thickness of moist vermiculite, and germinated in the dark at 25 °C for 4 days. The seedlings were then removed and placed in two trays (46 × 56 cm) and sufficient aqueous $CuCl_2$ (3×10^{-3}M) was added to cover the roots. After a period of 8 h in the dark, the inducer solution was removed, the seedlings washed with water, and the labelled precursor solution was administered to the roots. Precursors were fed as their sodium salts by dissolving them in aqueous NaOH (0.5M; 250 ml) and adding phosphate buffer (0.1M, pH 7.0; 600 ml) and water (950 ml). The seedlings were grown on in the dark for 16 h, after which they were homogenized in a blender. The slurry and residual feeding solution was then poured into hot EtOH (4 l), cooled, and filtered. The tissue was re-extracted with hot EtOH (3 × 3 l) and the combined extracts evaporated to dryness. After addition of water (0.8 l), the solution was extracted with ether (1 × 1 l, then 4 × 0.8 l) and the extracts bulked and evaporated. The crude extract was separated by t.l.c. (hexane–ethyl acetate, 3:2), and bands corresponding to markers of formononetin and medicarpin + maackiain were removed and eluted (AnalaR acetone).

The formononetin band was purified further by t.l.c. (chloroform–methanol, 2:1) and quantified by u.v. absorption of an EtOH solution, $\lambda_{max.}$ 248 nm (log ε 4.44).[11] The product was acetylated in dry pyridine (20 ml) with Ac_2O (2 ml) overnight at room temperature. The reaction mixture was poured into water, extracted with ethyl acetate (3 × 50 ml), washed with 10% HCl (2 × 100 ml), and finally water. T.l.c. of the evaporated extracts (hexane–acetone, 2:1) afforded formononetin acetate, m.p. 170—171 °C (lit.,[30] 169—170 °C), which was recrystallized (× 5) to constant specific activity from aqueous MeOH; *m/z* (%) 311 (12, $M^+ + 1$), 310 (61, M^+), 269 (18), 268 (100), 267 (40), 253 (14), 132 (48), 117 (10), and 89 (11); δ_H [250 MHz, $(CD_3)_2SO$] 2.34 (3 H, s, OAc), 3.79 (3 H, s, OMe), 7.04 (2 H, d, *J* 8.8 Hz, 3′,5′-H), 7.55 (2 H, d, *J* 8.7 Hz, 2′,6′-H), 7.57 (1 H, d, *J* 2.1 Hz, 8-H), 7.34 (1 H, dd, *J* 8.6, 2.1 Hz, 6-H), 8.19 (1 H, d, *J* 8.7 Hz, 5-H), and 8.51 (1 H, s, 2-H); δ_C [62.89 Mz, $(CD_3)_2SO$] 20.79 (acetyl Me), 55.10 (OMe), 111.29 (C-8), 113.64 (C-3′,5′), 119.90 (C-6), 121.57 (C-4a), 123.62 (C-3), 123.71 (C-1′), 126.90 (C-5), 130.01 (C-2′,6′), 153.98 (C-2), 154.33 (C-7), 156.02 (C-8a), 159.11 (C-4′), 168.52 (acetyl CO), and 174.67 (C-4).

The medicarpin/maackiain fraction was purified further by t.l.c. (hexane–acetone, 2:1), after which the two compounds were separated by gel filtration[31] (Sephadex LH-20, column 3 × 45 cm, solvent EtOH, flow rate 60 ml/h, u.v. detector 280 nm). Medicarpin was collected in the 270—360 ml eluate, and maackiain in the 410—515 ml eluate. The unresolved mixture in the 360—410 ml eluate was rechromatographed until complete separation was achieved. Fractions containing pure medicarpin or maackiain were separately combined and the pterocarpan content was determined by u.v. absorption of EtOH solutions, medicarpin, $\lambda_{max.}$ 284 nm (log ε 3.94); maackiain, $\lambda_{max.}$ 308 nm (log ε 3.87).[11] The pterocarpans were acetylated and purified as above to yield (−)-medicarpin acetate, m.p. 121—122 °C (lit.,[32] 122—123 °C) and (−)-maackiain acetate, m.p. 177—178 °C (lit.,[30] 176—177 °C), recrystallized (× 5) to constant specific activity from aqueous MeOH.

Medicarpin acetate: *m/z* (%) 313 (8, $M^+ + 1$), 312 (41, M^+), 271 (18), 270 (100), 269 (42), 255 (27), and 148 (16); δ_H [250 MHz, $(CD_3)_2CO$] 2.24 (3 H, s, $COCH_3$), 3.66 (1 H, m, 6a-H), 3.70 (1 H, m, 6-H-*pro-S*), 3.74 (3 H, s, OMe), 4.36 (1 H, m, 6-H-*pro-R*), 5.61 (1 H, d, *J* 6.7 Hz, 11a-H), 6.40 (1 H, d, *J* 2.2 Hz, 10-H), 6.49 (1 H, dd, *J* 8.2, 2.2 Hz, 8-H), 6.69 (1 H, d, *J* 2.2 Hz, 4-H), 6.83 (1 H, dd, *J* 8.3, 2.2 Hz, 2-H), 7.27 (1 H, d, *J* 8.2 Hz, 7-H), and 7.55 (1 H, d, *J* 8.4 Hz, 1-H); δ_C [62.89 MHz, $(CD_3)_2CO$] 20.91 (acetyl Me), 40.40 (C-6a), 55.75 (OMe), 67.28 (C-6), 78.75 (C-11a), 97.35 (C-10), 107.24 (C-8), 111.32 (C-4), 116.03 (C-2), 119.18, 120.01 (C-6b, 1a), 125.83 (C-7), 132.73 (C-1), 152.83 (C-3), 157.21 (C-4a), 161.62 (C-10a), 162.20 (C-9), and 169.32 (acetyl CO).

Maackiain acetate: *m/z* (%) 327 (8, $M^+ + 1$), 326 (41, M^+), 285 (18), 284 (100), 283 (22), 267 (10), 162 (16), and 134 (11); δ_H [250 MHz, $(CD_3)_2CO$] 2.24 (3 H, s, $COCH_3$), 3.65 (1 H, m, 6a-H), 3.74 (1 H, *ca.* t, *J* 9.8 Hz, 6-H-*pro-S*), 4.37 (1 H, *ca.* dd, *J* 9.9, 3.6 Hz, 6-H-*pro-R*), 5.58 (1 H, d, *J* 6.6 Hz, 11a-H), 5.91 (1 H, d, *J* 0.9 Hz, OCH_2O), 5.94 (1 H, d, *J* 0.9 Hz, OCH_2O), 6.42 (1 H, s, 10-H), 6.68 (1 H, d, *J* 2.3 Hz, 4-H), 6.82 (1 H, dd, *J* 8.4, 2.3 Hz, 2-H), 6.92 (1 H, s, 7-H), and 7.52 (1 H, d, *J* 8.3 Hz, 1-H); δ_C [62.89 MHz, $(CD_3)_2CO$] 20.93 (acetyl Me), 41.13 (C-6a), 67.22 (C-6), 78.78 (C-11a), 94.06 (C-10), 102.21 (OCH_2O), 105.89 (C-7), 111.35 (C-4), 116.06 (C-2), 119.15, 119.27 (C-6b, C-1a), 132.71 (C-1), 142.77 (C-8), 149.12 (C-9), 152.91 (C-3), 155.20 (C-10a), 157.27 (C-4a), and 169.35 (acetyl CO).

Experiments using 30 g of red clover seeds were essentially scaled-down versions of the above. For tracer experiments using 2 g of seeds, techniques were as previously described.[11,14] These involved dilution of the isolated isoflavonoids with synthetic carrier and final purification *via* the methyl ethers.

[1,2-$^{13}C_2$]*Resacetophenone.*—Freshly sublimed resorcinol (1 g) and [1,2-$^{13}C_2$]acetyl chloride (92% ^{13}C at C-1, 91% at C-2; 0.5 g) were stirred at room temperature with anhydrous $AlCl_3$ (1.2 g) in dry nitrobenzene (8.5 ml) for 24 h. After decomposition of the $AlCl_3$ with ice-cold 10% HCl, the mixture was extracted with ethyl acetate (50 ml), and this solution was washed with 2% NaOH (3 × 75 ml). The basic washings were acidified with 10% HCl, extracted with ethyl acetate (3 × 100 ml), and the combined extracts washed with water and evaporated. Recrystallization of the residue from water yielded [1,2-$^{13}C_2$]resacetophenone (0.9 g, 65%), identical with authentic material.

2′,4,4′-*Trihydroxy*[carbonyl,α-$^{13}C_2$]*chalcone* (**12**).—[1,2-$^{13}C_2$]Resacetophenone (0.85 g) was heated at 100 °C with 4-hydroxybenzaldehyde (0.7 g), EtOH (0.7 ml) and aqueous KOH (10 g KOH/7 ml H_2O; 5.2 ml) in a stoppered flask for 1 h, then left at room temperature overnight. The mixture was acidified with concentrated HCl and the precipitate (0.88 g) filtered off and washed with water. Unchanged resacetophenone was recovered from the filtrate by extraction and t.l.c. (chloroform–methanol, 9:1) and re-treated as above to yield further chalcone (0.42 g). The bulked product was purified by recrystallization from aqueous MeOH to give the required chalcone (1.21 g, 85%), identical with authentic material; δ_C [62.89 Mz, $(CD_3)_2CO$] 103.77 (C-3′), 108.74 (C-5′), 114.42 (C-1′), 116.74 (C-3,5), 118.13 (C-α), 127.41 (C-1), 131.62 (C-2, 6), 133.11 (C-6′), 145.11 (C-β), 160.85 (C-4), 165.49 (C-2′), 167.38 (C-4′), and 192.73 (CO); labelled material had satellite signals for 118.13 and 192.73 (*J* 22.5 Hz).

4-*Hydroxy*[formyl-^{2}H]*benzaldehyde.*—Perchloric acid (60%; 6 ml) was slowly added to 4-benzyloxybenzaldehyde (6.8 g) in morpholine (50 ml) with stirring, and the solution was heated at 60 °C for 1 h. KCN (2.75 g) in water (6 ml) was added and the mixture was stirred at 90 °C for a further 1 h. Addition of ice-cold water (150 ml) to the cooled mixture precipitated a yellow

solid which was recrystallized from chloroform–light petroleum (b.p. 60—80 °C) to give α-(4-benzyloxyphenyl)-α-morpholinoacetonitrile (9.5 g, 96%), m.p. 136—138 °C; δ_H (60 MHz, $CDCl_3$) 2.54 (4 H, t, *J* 5 Hz, NCH_2CH_2O), 3.75 (4 H, t, *J* 5 Hz, NCH_2CH_2O), 4.76 (1 H, s, CHCN), 5.10 (2 H, s, CH_2Ph), 7.08 (2 H, d, *J* 9 Hz, 3′,5′-H), 7.41 (5 H, s, CH_2Ph), and 7.55 (2 H, d, *J* 9 Hz, 2′,6′-H). The morpholinoacetonitrile derivative (4 g) was dissolved in dry DMF (60 ml) under dry, O_2-free N_2. The stirred solution was treated with sodium hydride (50% mineral oil suspension; 2 g) at 40 °C for 1 h to generate the yellow benzylic anion. Slow addition of ^2H_2O (99.8% ^2H; 2 ml) was followed by acidification with thionyl chloride (5 ml). After 5 min, the mixture was diluted with ice–water (400 ml), extracted with ethyl acetate (3 × 200 ml), and washed with water. The extract was evaporated and the residue crystallized from chloroform–petroleum to yield α-(4-benzyloxyphenyl)-α-morpholino[α-^{2}H]-acetonitrile (2.5 g). The deuteriation was then repeated to maximize deuterium content (*ca.* 95% from n.m.r.), yield 1.6 g (40%). This material was hydrolysed in a mixture of 2M-HCl (30 ml) and EtOH (90 ml) at 70 °C for 2 h. The solution was concentrated, extracted with ethyl acetate (3 × 100 ml), and the extracts washed with water. The residue was recrystallized from aqueous EtOH to afford 4-benzyloxy[*formyl*-^{2}H]benzaldehyde (1.05 g, 95%), which was debenzylated by heating and stirring with HOAc (100 ml) and concentrated HCl (50 ml) at 70 °C overnight. The mixture was poured into water, extracted with ethyl acetate (3 × 100 ml), and the extracts washed with saturated aqueous $NaHCO_3$ (2 × 100 ml), and then water. After evaporation, the residue was purified by t.l.c. (chloroform–methanol, 10:1) to give 4-hydroxy[*formyl*-^{2}H]-benzaldehyde (0.42 g, 76%); δ_H [60 MHz, $(CD_3)_2CO$] 9.91 (*ca.* 5%, CHO residue).

4-*Hydroxy*[formyl,3,5-$^2H_3$]*benzaldehyde*.—4-Hydroxy-[*formyl*-^{2}H]benzaldehyde (0.39 g), ^2H_2O (2.5 ml), and Et_3N (0.45 ml) were heated in a N_2-flushed Reactivial at 100 °C for 6 days. After cooling, the mixture was transferred to water (50 ml), acidified with 10% HCl, and extracted with ethyl acetate (3 × 30 ml). The combined extracts were washed with water, evaporated, and dried to yield the labelled aldehyde (0.37 g). The exchange was then repeated, yield 0.34 g (87%); δ_H [60 MHz, $(CD_3)_2CO$] 9.91 (*ca.* 5%, CHO residue), 6.91 (about 7%, 3,5-H residue).

2′,4,4′-*Trihydroxy*[β,3,5-$^2H_3$]*chalcone* (**21**).—Resacetophenone (0.24 g) and 4-hydroxy[*formyl*,3,5-$^2H_3$]benzaldehyde (0.20 g) were heated with ethanol (0.2 ml) and aqueous KOH (1.5 ml) as above. The chalcone was isolated, recrystallized, and further purified by t.l.c. (chloroform–methanol, 7:1), yield 0.17 g (51%); δ_H [60 MHz; $(CD_3)_2CO$] 7.76 (*ca.* 5%, β-H residue), 6.94 (*ca.* 7%, 3,5-H residue).

4-*Methoxy*[3,5-$^2H_2$]*benzaldehyde*.—4-Hydroxy[3,5-$^2H_2$]-benzaldehyde (prepared as above by $Et_3N/^2H_2O$ exchange of 4-hydroxybenzaldehyde, 1.7 g) was stirred and heated under reflux with anhydrous K_2CO_3 (7 g) and MeI (5 ml) in dry acetone (40 ml) for 1.5 h. The mixture was filtered and the inorganic material washed with acetone. The combined filtrates were evaporated to give a yellow oil which was purified by t.l.c. [light petroleum (b.p. 60—80 °C)–ethyl acetate, 9:1], yield 1.6 g (84%). The product was stored under N_2; δ_H (60 MHz, $CDCl_3$) 6.96 (*ca.* 5%, 3,5-H residue).

4′-*Benzyloxy*-2′-*hydroxy*-4-*methoxy*[3,5-$^2H_2$]*chalcone* (**24**).—4′-Benzyloxy-2′-hydroxyacetophenone (2 g) and 4-methoxy[3,5-$^2H_2$]benzaldehyde (1.5 g) were dissolved in EtOH (60 ml), a solution of KOH (15 g) in water (15 ml) was added, and the solution was stirred at room temperature overnight. The mixture was poured into cold water, acidified with concentrated HCl and extracted with ethyl acetate (3 × 100 ml). The extracts were washed with water and evaporated. The residue was recrystallized from chloroform–methanol, yield 1.6 g (54%), identical with authentic material;[22] δ_H (60 MHz, $CDCl_3$) 6.91 (*ca.* 5%, 3,5-H residue).

7-O-*Benzyl*[3′,5′-$^2H_2$]*formononetin* (**23**).—4′-Benzyloxy-2′-hydroxy-4-methoxy[3,5-$^2H_2$]chalcone (1.3 g) was acetylated in dry pyridine (30 ml) with Ac_2O (3 ml) at room temperature overnight. The mixture was poured into water, extracted with ethyl acetate (3 × 50 ml), and the extracts washed with 10% HCl (2 × 100 ml) and then water. The extracts were evaporated to dryness to yield the chalcone acetate as a yellow oil. Without further purification, the acetate was dissolved with warming in MeOH (240 ml) and stirred at room temperature overnight with $Tl(NO_3)_3 \cdot 3H_2O$ (1.65 g). Solid KOH (3.3 g) was added, and the mixture stirred for a further 1 h. After neutralization with concentrated HCl, 10% HCl (27 ml) was added, and the mixture was heated under reflux for 2 h. The hot solution was filtered to remove inorganic material, and the filtrate concentrated and poured into water. Extraction with ethyl acetate (3 × 100 ml) and evaporation of the extracts afforded a residue which on recrystallization from MeOH gave the isoflavone (0.95 g, 73%), identical with authentic material;[22] δ_H (60 MHz, $CDCl_3$) 6.72 (*ca.* 5%, 3′,5′-H residue).

[3′,5′-$^2H_2$]*Formononetin* (**22**).—7-*O*-Benzyl[3′,5′-$^2H_2$]formononetin (0.9 g) was debenzylated by stirring and heating under reflux with cyclohexene (10 ml) and palladium hydroxide–charcoal catalyst (20%; 225 mg) in EtOH (20 ml) for 1.5 h. The hot mixture was filtered and the catalyst washed thoroughly with hot EtOH. The filtrates were evaporated and the residue recrystallized from MeOH to afford [3′,5′-$^2H_2$]formononetin (0.63 g, 93%), identical with authentic material; δ_H [60 MHz $(CD_3)_2CO$–$(CD_3)_2SO$] 6.78 (*ca.* 5%, 3′,5′-H residue).

(±)-7-*Benzyloxy*-4′-*methoxy*[2-$^2H_2$]*isoflavanone* (**31**).—4-Methoxybenzyl 4-benzyloxy-2-hydroxyphenyl ketone[33] (0.84 g) was stirred and heated under reflux with anhydrous K_2CO_3 (3.3 g) and [$^2H_2$]methylene iodide[34] (0.59 ml) in dry acetone (40 ml) for 160 h. The mixture was filtered and the inorganic material washed with acetone. The filtrate was evaporated and the residue purified by t.l.c. (hexane–acetone, 4:1). The isoflavanone (253 mg, 29%) was obtained by recrystallization from MeOH; δ_H (60 MHz, $CDCl_3$) 3.78 (3 H, s, OMe), 3.84 (1 H, s, 3-H), 4.51 (*ca.* 5%, 2-H residue), 5.02 (2 H, s, OCH_2Ph), 6.44 (2 H, m, 6,8-H), 6.72 (2 H, d, *J* 9 Hz, 3′,5′-H), 7.08 (2 H, d, *J* 9 Hz, 2′,6′-H), 7.29 (5 H, s, OCH_2Ph), and 7.77 (1 H, d, *J* 9 Hz, 5-H).

(±)-7-*Hydroxy*-4′-*methoxy*[2-$^2H_2$]*isoflavanone* (**30**).—(±)-7-Benzyloxy-4′-methoxy[2-$^2H_2$]isoflavanone (253 mg) was debenzylated by stirring and heating under reflux with cyclohexene (4 ml) and $Pd(OH)_2$–C catalyst (20%; 90 mg) in EtOH (8 ml) for 1.5 h. The hot mixture was filtered, the catalyst washed with hot EtOH, and the filtrates evaporated. The product was obtained by t.l.c. (chloroform–isopropyl alcohol, 10:1) and purified by recrystallization from MeOH; yield 162 mg (85%), m.p. 197—199 °C (lit.,[35] 197 °C), δ_H [250 MHz, $(CD_3)_2CO$] 3.76 (3 H, s, OMe), 3.89 (1 H, s, 3-H), 4.64 (*ca.* 5%, 2-H residue), 6.41 (1 H, d, *J* 2.3 Hz, 8-H), 6.58 (1 H, dd, *J* 8.7, 2.4 Hz, 6-H), 6.90 (2 H, d, *J* 8.7 Hz, 3′,5′-H), 7.23 (2 H, d, *J* 8.5 Hz, 2′,6′-H), and 7.75 (1 H, d, *J* 8.7 Hz, 5-H).

(±)-4′,7-*Dibenzyloxy*[2-^{2}H]*flavanone* (**35**).—4-Benzyloxy-[*formyl*-^{2}H]benzaldehyde (4.6 g) and 4′-benzyloxy-2′-hydroxyacetophenone (4.6 g) were dissolved in warm EtOH (185 ml), a solution of KOH (46 g) in water (46 ml) was added, and the

mixture was stirred at room temperature overnight. The chalcone was precipitated by pouring the mixture into ice–water and acidifying with concentrated HCl. The precipitate was filtered off, washed, air-dried, and recrystallized from chloroform–methanol, yield 6 g (72%), m.p. 140—141 °C (lit.,[36] 139 °C). This chalcone (5 g) was dissolved with warming in MeOH (2.9 l), saturated aqueous sodium acetate (250 g/500 ml; 430 ml) was added, and the mixture was heated under reflux for 72 h. After concentration, the mixture was extracted with ethyl acetate (3 × 500 ml), washed with water, and evaporated. The residue was separated by column chromatography on Al_2O_3 (Woelm neutral alumina for dry column chromatography, 500 g), eluting with MeOH to afford the flavanone (3.5 g, 70%) which was further purified by recrystallization from MeOH, m.p. 125—127 °C (lit.,[18] m.p. 124—126 °C); δ_H (60 MHz, $CDCl_3$) 5.35 (*ca.* 6%, 2-H residue).

(±)-4′,7′-*Dibenzyloxy*[2,3-$^2H_3$]*flavanone* (**36**).—Alumina (Woelm 200 basic; 30 g) was stirred with ^2H_2O (5 ml) at room temperature for 1 h. 4′,7-Dibenzyloxy[2-^{2}H]flavanone (250 mg) in benzene (30 ml) was added and the slurry stirred at room temperature overnight. After filtration and evaporation, the flavanone (235 mg, 94%) was obtained by recrystallization from MeOH. The procedure was then repeated. δ_H (60 MHz, $CDCl_3$) 5.35 (*ca.* 6%, 2-H residue), 2.85 (*ca.* 8%, 3-H residue).

2′,4,4′-*Trihydroxy*[α,β-$^2H_2$]*chalcone* (**33**).—4′,7-Dibenzyloxy[2,3-$^2H_3$]flavanone (2.7 g) was dissolved in dry dioxane (72 ml) and stirred with 5% NaO^2H in ^2H_2O (32 ml) at room temperature for 1 h. Ether (100 ml) was added to the reaction mixture and the alkali removed by washing with water. After evaporation of the ether, the residue was dried then debenzylated with BBr_3 (4 ml) in dry CH_2Cl_2 (270 ml) at room temperature for 2 h. The solvent and BBr_3 were evaporated, and water (200 ml) was added. The product was extracted with ethyl acetate (3 × 200 ml), washed with water, and evaporated. The residue was recrystallized from aqueous MeOH to yield 2′,4,4′-trihydroxy[α,β-$^2H_2$]chalcone (1.51 g, 95%); δ_H [250 MHz $(CD_3)_2CO$] 7.71 (*ca.* 6%, β-H residue), 7.55 (*ca.* 6% α-H residue).

Acknowledgements

We thank the Iraqi Government for financial support (to H. A. M. A.)

References

1 J. Ebel and K. Hahlbrock in 'The Flavonoids: Advances in Research,' eds. J. B. Harborne and T. J. Mabry, Chapman and Hall, London, 1982, p. 641.
2 H. Grisebach and N. Doerr, *Z. Naturforsch.*, 1959, **14b**, 802; 1960, **15b**, 284; H. Grisebach and L. Patschke, *Chem. Ber.*, 1960, **93**, 2326; H. Grisebach and G. Brandner, *Z. Naturforsch.*, 1961, **16b**, 2.
3 H. Grisebach and W. Barz, *Z. Naturforsch.*, 1963, **18b**, 466; 1964, **19b**, 596.
4 L. Crombie and M. B. Thomas, *J. Chem. Soc. C*, 1967, 1796; L. Crombie, C. L. Green, and D. A. Whiting, *ibid.*, 1968, 3029.
5 P. M. Dewick, *Phytochemistry*, 1978, **17**, 249.
6 W. Barz and H. Grisebach, *Z. Naturforsch.*, 1967, **22b**, 627; J. Ebel, H. Achenbach, W. Barz, and H. Grisebach, *Biochim. Biophys. Acta*, 1970, **215**, 203.
7 E. Wong, *Phytochemistry*, 1968, **7**, 1751; E. Wong and H. Grisebach, *ibid.*, 1969, **8**, 1419.
8 H. Grisebach and W. D. Ollis, *Experientia*, 1961, **17**, 4; H. Grisebach in 'Chemistry and Biochemistry of Plant Pigments,' ed. T. W. Goodwin, Academic Press, London, 1965, p. 279; E. Wong, *Fortschr. Chem. Org. Naturst.*, 1970, **28**, 1; W. D. Ollis, K. L. Ormand, B. T. Redman, R. J. Roberts, and I. O. Sutherland, *J. Chem. Soc. C*, 1970, 125; A. Pelter, R. S. Ward, and M. Balasubramanian, *J. Chem. Soc., Chem. Commun.*, 1976, 151.
9 J. L. Ingham, *Fortschr. Chem. Org. Naturst.*, 1983, **43**, 1; P. M. Dewick in 'The Flavonoids: Advances in Research,' eds. J. B. Harborne and T. J. Mabry, Chapman and Hall, London, 1982, p. 535.
10 W. Barz and H. Grisebach, *Z. Naturforsch.*, 1966, **21b**, 1113; H. A. M. Al-Ani and P. M. Dewick, *Phytochemistry*, 1980, **19**, 2337.
11 P. M. Dewick, *Phytochemistry*, 1975, **14**, 979.
12 A. Pelter, J. Bradshaw, and R. F. Warren, *Phytochemistry*, 1971, **10**, 835.
13 H. Grisebach and W. Barz, *Chem. Ber.*, 1964, **97**, 1688; H. O. House, *J. Am. Chem. Soc.*, 1954, **76**, 1235.
14 P. M. Dewick, *Phytochemistry*, 1977, **16**, 93.
15 P. M. Dewick, and D. Ward, *Phytochemistry*, 1978, **17**, 1751.
16 D. J. Bennett, G. W. Kirby, and V. A. Moss, *J. Chem. Soc. C*, 1970, 2049.
17 G. W. Kirby and L. Ogunkoya, *J. Chem. Soc. C*, 1965, 6914.
18 H. Grisebach and H. Zilg, *Z. Naturforsch.*, 1968, **23b**, 494.
19 J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, 1972, **28**, 1120.
20 L. Farkas, A. Gottsegen, M. Nogradi, and S. Antus, *J. Chem. Soc., Perkin Trans. 1*, 1974, 305.
21 S. Hanessian, T. J. Liak, and B. Vanasse, *Synthesis*, 1981, 396.
22 P. M. Dewick and M. Martin, *Phytochemistry*, 1979, **18**, 597.
23 H. Suginome and T. Iwadare, *Bull. Chem. Soc. Jpn.*, 1960, **33**, 567.
24 S. K. Aggarwal, S. K. Grover, and T. R. Seshadri, *Indian J. Chem.*, 1972, **10**, 804.
25 G. Zemplen, R. Bognar, and L. Mester, *Chem. Ber.*, 1942, **75**, 1432.
26 K. Hahlbrock, H. Zilg, and H. Grisebach, *Eur. J. Biochem.*, 1970, **15**, 13.
27 P. M. Dewick and D. Ward, *J. Chem. Soc., Chem. Commun.*, 1977, 338; S. W. Banks, M. J. Steele, D. Ward, and P. M. Dewick, *ibid.*, 1982, 157.
28 M. Martin and P. M. Dewick, *Phytochemistry*, 1980, **19**, 2341.
29 L. Crombie, P. M. Dewick, and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1285.
30 D. M. X. Donnelly, J. C. Thompson, W. B. Whalley, and S. Ahmad, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1737.
31 S. W. Banks and P. M. Dewick, *Phytochemistry*, 1983, **22**, 1591.
32 T. B. H. McMurray, E. Martin, D. M. X. Donnelly, and J. C. Thompson, *Phytochemistry*, 1972, **11**, 3283.
33 H. S. Mahal, H. S. Rai, and K. Venkataraman, *J. Chem. Soc.*, 1934, 1769.
34 F. Fisher and D. E. Applequist, *J. Labelled Comp.*, 1969, **5**, 86.
35 N. Inoue, *Sci. Rep. Tohoku Univ. Ser. 1*, 1961, **45**, 63 (*Chem. Abstr.*, 1963, **58**, 5619).
36 H. S. Mahal, H. S. Rai, and K. Venkataraman, *J. Chem. Soc.*, 1935, 866.

Received 4*th May* 1984; *Paper* 4/712