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BIOSYNTHESIS OF PTEROCARPAN, ISOFLAVAN AND COUMESTAN METABOLITES OF *MEDICAGO SATIVA*: THE ROLE OF AN ISOFLAV-3-ENE

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Key Word Index—*Medicago sativa*; Leguminosae; lucerne; biosynthesis; phytoalexin; pterocarpan; isoflavan; coumestan; demethylhomopterocarpin; vestitol; sativan; 9-*O*-methylcoumestrol.

Abstract—Feeding experiments in $CuCl_2$- and UV-treated lucerne (*Medicago sativa*) seedlings have shown that demethylhomopterocarpin-[6a-^{3}H, Me-^{14}C] is incorporated into vestitol and sativan without any loss of ^{3}H label, and vestitol-[3-^{3}H, Me-^{14}C] is similarly incorporated into demethylhomopterocarpin and sativan with retention of the ^{3}H:^{14}C ratio. Thus, an isoflav-3-ene intermediate in the interconversion of demethylhomopterocarpin and vestitol is excluded. 7,2′-Dihydroxy-4′-methoxyisoflav-3-ene-[Me-^{14}C] was not incorporated into the three phytoalexins, but was an excellent precursor of 9-*O*-methylcoumestrol, as also was 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one-[Me-^{14}C]. A biosynthetic pathway to coumestans via isoflav-3-enes and 3-arylcoumarins is proposed. A metabolic scheme in *M. sativa* interrelating eight classes of naturally occurring isoflavonoids is presented.

INTRODUCTION

Upon fungal infection, lucerne (*Medicago sativa*) produces three isoflavonoid phytoalexins (6a*R*, 11a*R*)-demethylhomopterocarpin (**8**), (3*R*)-vestitol (**4**) and (3*R*)-sativan (**7**) [1–3]. Lucerne seedlings synthesize the same three compounds when challenged by abiotic agents such as $CuCl_2$ and UV light, and this treatment also stimulates synthesis of the coumestan 9-*O*-methylcoumestrol (**10**) [4, 5]. Earlier feeding experiments [4, 5] have indicated that these four compounds are closely related biosynthetically and share common precursors such as formononetin (**1**), 7,2′-dihydroxy-4′-methoxyisoflavone (**2**) and 7,2′-dihydroxy-4′-methoxyisoflavanone (**3**). Vestitol and demethylhomopterocarpin appear to be produced simultaneously but are readily interconvertible in *M. sativa*, and an intermediate carbonium ion (**5**), derived from the isoflavone (**3**) via the isoflavanol (**6**) has been proposed [4]. Sativan is most probably derived by methylation of vestitol.

An alternative, uncharged compound, 7,2′-dihydroxy-4′-methoxyisoflav-3-ene (**9**) has also been considered [4] as a biosynthetic intermediate in the interconversion of the pterocarpan and isoflavan. In the light of recent reports concerning the isolation of isoflav-3-enes from nature [6–8], speculative roles [7, 9, 10] for these reactive isoflavonoids become more attractive. Therefore, a number of feeding experiments have been performed to establish the role, if any, of 7,2′-dihydroxy-4′-methoxyisoflav-3-ene in the biosynthesis of these four induced isoflavonoids from lucerne. A preliminary communication of this work has been published [11].

RESULTS

Feeding experiments

Although 2′-hydroxyisoflav-3-enes may readily be prepared by controlled acid treatment of pterocarpans [10], these compounds are highly reactive, especially in solution, and are thus poorly suited for feeding experiments to plants. To circumvent this problem, and to assess the role of the isoflav-3-ene in the interconversion of demethylhomopterocarpin and vestitol, a double-labelling experiment was employed. (±)-Demethylhomopterocarpin-[6a-^{3}H, Me-^{14}C] and (±)-vestitol-[3-^{3}H, Me-^{14}C] (^{3}H:^{14}C = 5.0, *ca* 0.5 mg) were separately fed as their Na salts in phosphate buffer to batches of 4-day-old lucerne seedlings (from 6 g dry seeds) previously treated with UV radiation for 0.5 hr and aq. $CuCl_2$ for a period of 8 hr [5]. After a metabolism period of 16 hr, the three phytoalexins and 9-*O*-methylcoumestrol were isolated by TLC, quantified by UV spectroscopy and diluted with synthetic carrier. Demethylhomopterocarpin and 9-*O*-methylcoumestrol were converted into their methyl ethers, and vestitol and sativan were acetylated. These derivatives were then purified to constant specific activity and counted.

The results of the feeding experiments are summarized in Table 1, and show that demethylhomopterocarpin was transformed into vestitol and sativan with essentially no change in the ^{3}H:^{14}C ratio, but virtually all the ^{3}H was lost on incorporation into the coumestan. Similarly, vestitol was converted into demethylhomopterocarpin and sativan without significant change in the ^{3}H:^{14}C ratio. Although incorporation of vestitol into 9-*O*-methylcoumestrol was very small by comparison, all ^{3}H was again lost. These data infer that an isoflav-3-ene cannot be involved in the pterocarpan-2′-hydroxyisoflavan interconversion, since such an intermediate would necessitate complete loss of the ^{3}H label.

Whilst the double-labelling experiment ruled out any involvement of the isoflav-3-ene in the demethylhomopterocarpin–vestitol interconversion, this was not conclusive evidence that the compound had no role in the biosynthetic route to these compounds. Accordingly, 7,2′-

Table 1. Incorporation of double-labelled demethylhomopterocarpin and vestitol into *Medicago sativa* isoflavonoids

Metabolite	(±)-Demethylhomopterocarpin-[6a-^{3}H, Me-^{14}C]*				(±)-Vestitol-[3-^{3}H, Me-^{14}C]*			
	Sp. act.† (dpm/mM)	^{3}H: ^{14}C	Dilution†	Incorp.† (%)	Sp. act.† (dpm/mM)	^{3}H: ^{14}C	Dilution†	Incorp.† (%)
Demethylhomopterocarpin	7.05×10^7	5.5	5.0	39	3.72×10^6	5.0	110	1.4
Vestitol	1.44×10^7	5.1	25	0.93	1.21×10^8	5.3	3.3	7.1
Sativan	7.79×10^6	5.2	46	0.97	1.12×10^7	5.2	35	0.83
9-*O*-Methylcoumestrol	4.17×10^6	0.1	85	0.21	8.09×10^5	0.1	490	0.04

* ^{3}H: ^{14}C = 5.0

†Specific activity, dilution and incorporation refer to ^{14}C. Incorporation figures are not corrected for possible utilization of only one enantiomer from racemic mixtures.

Table 2. Incorporation of Me-^{14}C-labelled isoflavonoids into *Medicago sativa* metabolites

Compound fed	Expt.	Demethylhomopterocarpin			Vestitol			Sativan			9-*O*-Methylcoumestrol		
		Sp. act. (dpm/mM)	Dilution	Incorp. (%)	Sp. act. (dpm/mM)	Dilution	Incorp. (%)	Sp. act. (dpm/mM)	Dilution	Incorp. (%)	Sp. act. (dpm/mM)	Dilution	Incorp. (%)
7,2′-Dihydroxy-4′-methoxyisoflav-3-ene	(i)	9.38×10^5	1230	0.06	4.03×10^6	285	0.03	1.19×10^6	970	0.01	5.49×10^8	2.1	4.1
	(ii)	9.29×10^5	1240	0.09	2.97×10^6	390	0.04	4.19×10^5	2750	0.006	5.51×10^8	2.1	5.3
7,2′-Dihydroxy-4′-methoxyisoflavone	(ii)	6.53×10^7	18	7.9	7.20×10^7	16	1.0	4.79×10^7	24	0.34	1.09×10^8	11	0.66
(±)-Demethylhomopterocarpin	(ii)	2.95×10^6	3.9	33	1.17×10^7	98	0.13*	3.58×10^7	32	0.41*	4.60×10^7	25	0.13*
7,2′-Dihydroxy-4′-methoxyisoflav-3-en-2-one	(ii)	6.25×10^5	1840	0.06	1.76×10^6	680	0.02	4.08×10^5	2820	0.007	3.05×10^8	3.8	2.2

* Not corrected for possible utilization of only one enantiomer.

dihydroxy-4′-methoxyisoflav-3-ene-[Me-^{14}C] was synthesized and tested as a precursor of the lucerne metabolites. The labelled material was administered in a 2-methoxyethanol–H_2O–Tween 20 feeding solution since the isoflav-3-ene underwent fairly rapid decomposition in the NaOH–phosphate buffer medium normally employed, the solution turning yellow within 1–2 hr, and decomposition being evident from the UV spectrum. In the former feeding solution, the isoflav-3-ene could be recovered unchanged after 16 hr. The isoflav-3-ene was fed immediately after purification to minimize further any decomposition. The results of the feeding experiment are shown in Table 2 [experiment (i)], and indicate that incorporation into the phytoalexins was minimal although this compound was an excellent precursor of 9-*O*-methylcoumestrol. The result was confirmed in a repeat feeding [Table 2, experiment(ii)]. In this experiment, comparative feedings were also made using 7,2′-dihydroxy-4′-methoxyisoflavone-[Me-^{14}C] (**2**), (±-demethylhomopterocarpin-[Me-^{14}C] and the 3-arylcoumarin 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one-[Me^{14}C] (**11**), and these results are also included in Table 2. Whilst the isoflavone was incorporated into all four metabolites as previously [4, 5], and demethylhomopterocarpin into the other three isoflavonoids (cf. Table 1), significant incorporation of label from the isoflav-3-ene and the 3-arylcoumarin was observed only in the case of 9-*O*-methylcoumestrol.

Synthetic studies

(±)-Demethylhomopterocarpin-[6a-^{3}H] was prepared by $NaBH_4$ reduction of 7,2′-dihydroxy-4′-methoxyisoflavanone-[3-^{3}H] obtained from the unlabelled isoflavanone by base-catalysed (Al_2O_3) exchange [12] with tritiated water. A preliminary experiment using D_2O indicated the location of the introduced label in the isoflavanone. The NMR spectrum of the unlabelled material showed a double doublet at δ 4.10 (J = 11, 5 Hz) assigned to H-3, and a double doublet at 4.40 (J = 11, 5 Hz) together with a triplet at 4.60 (J = 11 Hz) assigned to the two H-2 protons. Following deuteriation, the spectrum showed almost complete disappearance of the H-3 signal, and the H-2 signals collapsed to two doublets at δ 4.40 and 4.60 (J = 11 Hz). The demethylhomopterocarpin-[6a-^{3}H] was then mixed with an appropriate amount of demethylhomopterocarpin-[Me-^{14}C] to give a ^{3}H:^{14}C ratio of 5.0. Confirmation of the position of tritium labelling was obtained by converting a portion of the doubly-labelled pterocarpan into 7,2′-dihydroxy-4′-methoxyisoflav-3-ene by treatment with acid in EtOH. The ^{3}H:^{14}C ratio of the isoflav-3-ene produced was 0.073, indicating that 99% of the label was located at 6a and/or 11a of the pterocarpan, and almost certainly this would be at position 6a. (±)-Vestitol-[3-^{3}H, Me-^{14}C] was obtained by catalytic hydrogenation of the doubly-labelled pterocarpan. Although it was possible that catalytic hydrogenation could have caused some scrambling of the ^{3}H label [13], this was not checked at this stage. The loss of 98% of the ^{3}H label on biosynthetic incorporation into 9-*O*-methylcoumestrol, however, indicates that any randomization was very small.

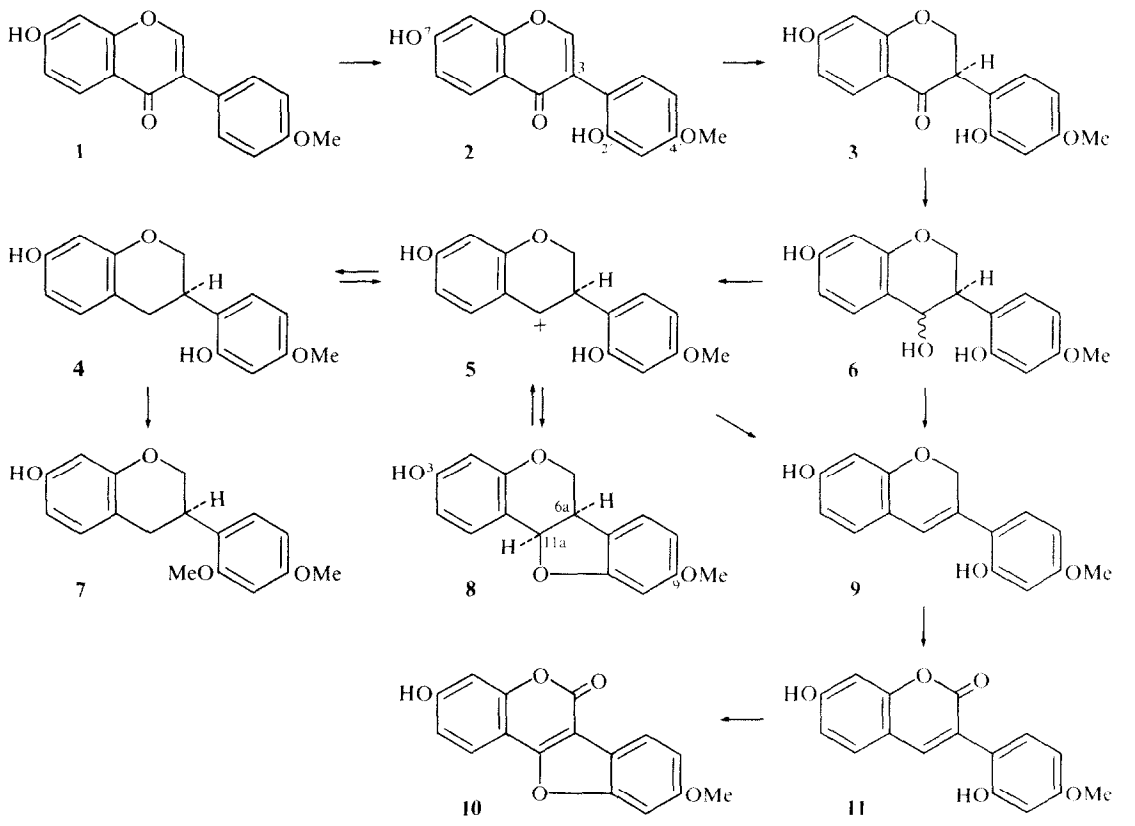
7,2′-Dihydroxy-4′-methoxyisoflav-3-ene-[Me-^{14}C] was synthesized from (±)-demethylhomopterocarpin-[Me-^{14}C] by acid treatment as above. The isoflav-3-ene could not be stored for long periods in EtOH solution; however, no 9-*O*-methylcoumestrol was detectable in the degradation products after 24 hr, indicating that auto-oxidation was not responsible for the observed incorporation of the isoflav-3-ene into the coumestan during the feeding experiments. 7,2′-Dihydroxy-4′-methoxyisoflav-3-en-2-one-[Me-^{14}C] was obtained from the isoflav-3-ene acetate by CrO_3 oxidation in pyridine [7], followed by deacetylation. No 9-*O*-methylcoumestrol was formed on leaving an EtOH solution of the 3-arylcoumarin in contact with air, similarly ruling out auto-oxidation during feeding experiments.

A chemical analogy for the biosynthetic isoflav-3-ene–coumestan conversion was provided by the reaction of (**9**) with DDQ in dioxan at room temperature, which produced 9-*O*-methylcoumestrol in 84% yield. Only traces of coumestan were observed on similar treatment of the 3-arylcoumarin (**11**) however, but a yield of 18% was obtained by heating with DDQ in refluxing benzene.

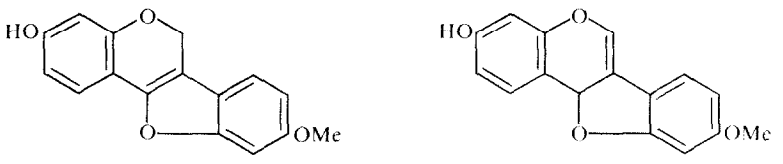
DISCUSSION

The complete retention of the ^{3}H:^{14}C ratio during the interconversion of demethylhomopterocarpin and vestitol demonstrates that no isoflav-3-ene or other 3,4-dehydro compound can be involved. A carbonium ion intermediate as previously postulated [4] is thus still favoured, and this is most likely produced from an isoflavanol (**6**) (Scheme 1). Support for this hypothesis comes from chemical studies, where photochemical fission of pterocarpans in MeOH gave 2′-hydroxy-4-methoxyisoflavans [14], which readily reverted to pterocarpans especially on treatment with acid. The 2′-hydroxyisoflavan–pterocarpan conversion was dependent on a good leaving group being present at C-4, thus favouring a carbonium ion mechanism. Attempted conversions of 2′-hydroxyisoflav-3-enes into pterocarpans failed. The only isoflavanol yet isolated from nature is a 2′-methoxy derivative, ambanol [15]; 2′-hydroxyisoflavanols are unlikely to be detected because of their ease of cyclization to pterocarpans via a carbonium ion.

These results are borne out by the negligible incorporation of ^{14}C-labelled 7,2′-dihydroxy-4′-methoxyisoflav-3-ene into the phytoalexins. Furthermore, the isoflav-3-ene would appear to play no other role in the biosynthetic pathway to these compounds. In contrast, it proved an extremely efficient precursor of 9-*O*-methylcoumestrol, as also was the 3-arylcoumarin. Earlier experiments [16, 17] have suggested that the biosynthetic route to coumestans may involve allylic oxidation of a pterocarp-6a-ene, e.g. **12**, produced by dehydration of a 2′-hydroxyisoflavanone, e.g. **3**. Isoflavanone (**3**) has been shown to be an excellent precursor of 9-*O*-methylcoumestrol in *M. sativa* [5]. Since pterocarp-6a-enes are so readily susceptible to auto-oxidation yielding coumestans [18–20], they have not been tested as precursors in feeding experiments. In the light of the present results, this route now seems less likely than a pathway involving dehydration of the isoflavanol (**6**) to isoflav-3-ene (**9**), allylic oxidation to the 3-arylcoumarin (**11**) and then ring cyclization by attack of the 2′-hydroxy onto the α,β-unsaturated lactone, followed by further oxidation to the coumestan (**10**). Isoflav-3-ene (**9**) may however, also be derived by loss of a proton from carbonium ion (**5**) and such a route would explain the significant incorporation of demethylhomopterocarpin into 9-*O*-methylcoumestrol (Tables 1 and 2). The proposed metabolic pathway in *M. sativa* is depicted in Scheme 1. Although several of the feeding experiments which have contributed to the above



Scheme 1



12 13

scheme have involved the use of racemic precursors, a number of the steps are in fact stereospecific [21] and these are indicated as such in the scheme. The scheme now interrelates 8 classes of naturally occurring isoflavonoids from the results of feeding experiments.

Both isoflav-3-enes and 3-arylcoumarins appear to be relatively rare in nature, although in the case of the isoflav-3-enes, this undoubtedly reflects their reactivity and difficulties associated with their isolation. Only four isoflav-3-enes, neorauflavene [6], sepiol [7], 2′-*O*-methylsepiol [7] and glabrene [8], and five 3-arylcoumarins, pachyrrhizin [22], neofolin [23], 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one [9], 7,2′-dihydroxy-4′5,-methylenedioxyisoflav-3-en-2-one [9] and glycyrin [24] have so far been reported in the literature. However, it is of particular interest that these compounds frequently co-occur with other classes of similarly substituted and biogenetically related isoflavonoids. Thus, **11**, the 3-arylcoumarin employed in these feeding experiments, has been isolated from *Dalbergia oliveri* [9] along with the 4′,5′-methylenedioxy analogue, and these two 3-arylcoumarins co-occur with the pterocarpans (6a*S*, 11a*S*)-demethylhomopterocarpin and (6a*S*,11a*S*)-maackiain, and the coumestans 9-*O*-methylcoumestrol and medicagol.

Although the biosynthetic pathway to coumestans probably proceeds from an isoflav-3-ene via a 3-arylcoumarin intermediate, the difference in reactivity of isoflav-3-ene (**9**) and 3-arylcoumarin (**11**) towards DDQ would suggest that **11** is not an intermediate in this chemical conversion of the isoflav-3-ene to the coumestan. This reaction may involve formation of an extended quinonemethide and cyclization of the 2′-hydroxy onto this system, producing pterocarp-6-ene (**13**) as an intermediate. Further oxidation of **13** would yield the coumestan [25]. Chemical oxidation of 2′-hydroxy-3-arylcoumarins to coumestans using lead tetraacetate instead of DDQ has been reported [26], but yields are similarly low.

EXPERIMENTAL

General. TLC was carried out using 0.5 mm layers of Si gel (Merck Kiesel gel GF_{254}) in the solvent systems: A, C_6H_6-

EtOAc–MeOH–petrol (bp 60–80°), 6:4:1:3; B, C_6H_6–EtOAc–MeOH–petrol (bp 60–80°), 6:4:1:6; C, C_6H_6–EtOAc, 32:1; D, $CHCl_3$–*iso*-PrOH, 10:1; E, $CHCl_3$–MeOH, 50:1; F, C_6H_6–EtOH, 9:1. Me_2CO (Analar) was used for elution of TLC zones. Radioactive samples were counted as previously [27], using toluene-[^{14}C] and toluene-[^{3}H] internal standards to measure efficiencies. The growing of plant material, isolation and purification of metabolites were as previously described [4, 5]. Feeding techniques were also as used earlier, except that 7,2′-dihydroxy-4′-methoxyisoflav-3-ene was dissolved in 2-methoxyethanol (0.5 ml) containing Tween 20 (1 drop), and the solution was then diluted with H_2O (5 ml).

Radiochemicals. The syntheses of (±)-demethylhomopterocarpin-[Me-^{14}C] (0.514 mCi/mM) and 7,2′-dihydroxy-4′-methoxyisoflavone-[Me-^{14}C] (0.518 mCi/mM) have been described [28].

(±)-*Demethylhomopterocarpin*-[6a-^{3}H, Me-^{14}C]. Al_2O_3 (Woelm 200 basic, 3 g) was stirred at room temp. for 1 hr with H_2O (0.5 ml) containing tritiated H_2O (40 μl, 0.2 Ci). 7,2′-Dihydroxy-4′-methoxyisoflavanone [28] (25 mg) in EtOAc (3 ml) was added, and the mixture was stirred at room temp. overnight. The mixture was filtered, the Al_2O_3 washed with EtOAc (3 ml), and the combined filtrates evapd to dryness. The residue was taken up in MeOH (10 ml) and the soln was then evapd to remove labile tritium. This procedure was repeated. (±)-7,2′-Dihydroxy-4′-methoxyisoflavanone-[3-^{3}H] was isolated from the residue by TLC (solvent A), then stirred at room temp. for 48 hr with THF (3 ml), EtOH (3 ml) and $NaBH_4$ (100 mg). After concn, the mixture was acidified and extracted with EtOAc (3 × 15 ml), and the extracts washed with H_2O. The extracts were evapd and the residue separated by TLC (solvent B) to give (±)-demethylhomopterocarpin-[6a-^{3}H] (1.41 mg). To this material (1.41 mg) was added (±)-demethylhomopterocarpin-[Me-^{14}C] (0.514 mCi/mM, 0.97 mg) and inactive (±)-demethylhomopterocarpin (0.65 mg), and the product was purified to constant sp. act. (^{3}H: 0.802 mCi/mM. ^{14}C: 0.160 mCi/mM: ^{3}H: ^{14}C = 5.0) by TLC (solvents C and D). A portion of this pterocarpan (0.2 mg) was heated under reflux with conc HCl (0.1 ml) in EtOH (5 ml) for 5 min. The solution was poured into H_2O, extracted with Et_2O (2 × 10 ml), and the extracts evapd. TLC (solvent E) of the residue gave 7,2′-dihydroxy-4′-methoxyisoflav-3-ene (^{3}H: ^{14}C = 0.073).

(±)-*Vestitol*-[3-^{3}H, Me-^{14}C]. (±)-Demethylhomopterocarpin-[6a-^{3}H, Me-^{14}C] (1.5 mg) was hydrogenated at room temp. overnight in EtOH (1.5 ml) over Pd/C catalyst (10%, 5 mg). The mixture was evapd, and the residue, including catalyst, was applied to the TLC plates which were developed in solvent A. (±)-Vestitol-[3-^{3}H, Me-^{14}C] (0.76 mg) was isolated, and purified to constant sp. act. (^{3}H: 0.878 mCi/mM, ^{14}C: 0.178 mCi/mM; ^{3}H: ^{14}C = 5.0) by TLC (solvents D and F).

7,2′-*Dihydroxy-4′-methoxyisoflav*-3-*ene*-[Me-^{14}C]. (±)-Demethylhomopterocarpin-[Me-^{14}C] (0.518 mCi/mM, 0.5 mg) in EtOH (3 ml) was heated under reflux with conc HCl (0.05 ml) for 5 min. The isoflav-3-ene was isolated as above and purified to constant sp. act. (0.518 mCi/mM) by TLC (solvents D and E). Yield 0.32 mg. Inactive material (recryst. $CHCl_3$) had mp 156–158°; λ_{max}^{EtOH} nm: 242 sh (log ε 4.11), 325 (4.23), 350 sh (3.95).

7,2′-*Dihydroxy*-4′-*methoxyisoflav*-3-*en*-2-*one*-[Me-^{14}C]. 7,2′-Dihydroxy-4′-methoxyisoflav-3-ene-[Me-^{14}C] was prepared as above from (±)-demethylhomopterocarpin-[Me-^{14}C] (1 mg) and then acetylated (Py–Ac_2O, room temp. overnight). The acetate was purified by TLC (solvent B). Inactive material (recryst. MeOH) had mp 83–85°; λ_{max}^{EtOH} nm: 293, 321; ^{1}H NMR (60 MHz, $CDCl_3$, TMS): δ 2.23 (6 H, *s*, OAc), 3.76 (3 H, *s*, OMe), 4.87 (2 H, *d*, *J* = 1 Hz, H-2), 6.50 (1 H, *d*, *J* = 1 Hz, H-4), 6.59 (3 H, *m*, H-3′, 5′, 8), 6.80 (1 H, *dd*, *J* = 9, 2 Hz, H-6), 6.97 (1 H, *d*, *J* = 9 Hz, H-6′), 7.22 (1 H, *d*, *J* = 9 Hz, H-5). The acetate was heated at 60° with CrO_3 (5 mg) in Py (2 ml) for 2 hr. The mixture was poured into H_2O, extracted with EtOAc (3 × 10 ml), the extracts washed with 10% HCl (2 × 10 ml) and finally H_2O. Evapn of the extracts and TLC (solvent B) of the residue gave 7,2′-diacetoxy-4′-methoxyisoflav-3-en-2-one-[Me-^{14}C]. Inactive material (recryst. MeOH) had mp 157–159° (lit. [9] 159–161°); λ_{max}^{EtOH} nm: 233, 283, 325; ^{1}H NMR (60 MHz, $CDCl_3$, TMS): δ 2.15 (3 H, *s*, OAc), 2.31 (3 H, *s*, OAc), 3.79 (3 H, *s*, OMe), 6.70–7.50 (6 H, *m*, aromatics), 7.58 (1 H, *s*, H-4). The acetate was deacetylated by stirring with NaOH (50 mg) in EtOH (4 ml) at room temp. for 1 hr. The mixture was concd, diluted with H_2O, acidified and extracted with EtOAc (2 × 10 ml). The extracts were evapd and the residue separated by TLC (solvent A) to give 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one-[Me-^{14}C] (0.31 mg) which was purified to constant sp. act. (0.518 mCi/mM) by TLC (solvents B and D). Inactive material (recryst. MeOH) had mp 254–258° (lit. [9] 258–260°); λ_{max}^{EtOH} nm: 242 (log ε = 3.99), 285 sh (3.77), 343 (4.20); ^{1}H NMR (60 MHz, $CDCl_3$, TMS): δ 3.75 (3 H, *s*, OMe), 6.70–7.30 (6 H, *m*, aromatics), 7.53 (1 H, *s*, H-4); MS (probe, 70 eV) *m/e* (rel. int.): 285 (15), 284 (100), 256 (8), 241 (48).

DDQ oxidation of 7,2′-*dihydroxy*-4′-*methoxy-isoflav*-3-*ene and* -*isoflav*-3-*en*-2-*one.* 7,2′-Dihydroxy-4′-methoxyisoflav-3-ene (9.9 mg) was treated with DDQ (25 mg) in 1,4-dioxan (3 ml) at room temp. overnight. The ppt. formed was filtered off, washed with dioxan and recryst. from THF–MeOH to yield 9-*O*-methylcoumestrol (6.6 mg), identical to authentic material [28]. Evapn of the mother-liquors followed by TLC (solvent A) afforded further product (1.8 mg).

7,2′-Dihydroxy-4′-methoxyisoflav-3-en-2-one (5 mg) was left at room temp. overnight with DDQ (15 mg) in 1,4-dioxan (3 ml). Analytical TLC (solvent A) of the reaction mixture showed only a trace of 9-*O*-methylcoumestrol. The reaction was repeated by heating the isoflav-3-en-2-one (5 mg) under reflux with DDQ (15 mg) in C_6H_6 (5 ml) for 16 hr. After evapn, the residue was separated by TLC (solvent A) and the 9-*O*-methylcoumestrol (1.0 mg) quantified by UV absorption [5].

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REFERENCES

1. Smith, D. G., McInnes, A. G., Higgins, V. J. and Millar, R. L. (1976) *Physiol. Plant Pathol.* **1**, 41.
2. Ingham, J. L. and Millar, R. L. (1973) *Nature* **242**, 125.
3. Ingham, J. L. (1979) *Biochem. Syst. Ecol.* **7**, 29.
4. Dewick, P. M. and Martin, M. (1979) *Phytochemistry* **18**, 591.
5. Dewick, P. M. and Martin, M. (1979) *Phytochemistry* **18**, 597.
6. Brink, A. J., Rall, G. J. H. and Engelbrecht, J. P. (1974) *Tetrahedron* **30**, 311.
7. Jurd, L. and Manners, G. D. (1977) *J. Agric. Food Chem.* **25**, 723.
8. Kinoshita, T., Saitoh, T. and Shibata, S. (1976) *Chem. Pharm. Bull.* **24**, 991.
9. Donnelly, D. M. X. and Kavanagh, P. J. (1974) *Phytochemistry* **13**, 2587.
10. Bevan, C. W. L., Birch, A. J., Moore, B. and Mukerjee, S. K. (1964) *J. Chem. Soc.* 5991.
11. Martin, M. and Dewick, P. M. (1978) *Tetrahedron Letters* 2341.
12. Grisebach, H. and Zilg, H. (1968) *Z. Naturforsch. Teil B* **23**, 494.
13. Evans, E. A. (1974) *Tritium and its Compounds*, 2nd edn, p. 564. Butterworths, London.
14. Van der Merwe, P. J., Rall, G. J. H. and Roux, D. G. (1978) *J. Chem. Soc. Chem. Commun.* 224.

15. Oberholzer, M. E., Rall, G. J. H. and Roux, D. G. (1977) *Tetrahedron Letters* 1165.
16. Dewick, P. M., Barz, W. and Grisebach, H. (1970) *Phytochemistry* **9**, 775.
17. Berlin, J., Dewick, P. M., Barz, W. and Grisebach, H. (1972) *Phytochemistry* **11**, 1689.
18. Dewick, P. M., Barz, W. and Grisebach, H. (1969) *Chem. Commun.* 466.
19. Ferreira, D., Brink, C. v. d. M. and Roux, D. G. (1971) *Phytochemistry* **10**, 1141.
20. Ferreira, M. A., Moir, M. and Thomson, R. H. (1974) *J. Chem. Soc. Perkin Trans* **1**, 2429.
21. Dewick, P. M. and Ward, D. (1977) *J. Chem. Soc. Chem. Commun.* 338.
22. Simonitsch, E., Frei, H. and Schmid, H. (1957) *Monatsh. Chem.* **88**, 541.
23. Brink, C. v. d. M., Nel, W., Rall, G. J. H., Weitz, J. C. and Pachler, K. G. R. (1966) *J. S. Afr. Chem. Inst.* **19**, 24.
24. Kinoshita, T., Saitoh, T. and Shibata, S. (1978) *Chem. Pharm. Bull.* **26**, 135.
25. Bowyer, W. J., Chatterjea, J. N., Dhoubhabel, S. P., Handford, B. O. and Whalley, W. B. (1964) *J. Chem. Soc.* 4212.
26. Kurosawa, K. and Nogami, K. (1976) *Bull. Chem. Soc. Jpn* **49**, 1955.
27. Dewick, P. M. (1975) *Phytochemistry* **14**, 979.
28. Dewick, P. M. (1977) *Phytochemistry* **16**, 93.