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BIOSYNTHESIS OF THE ISOFLAVAN ISOMUCRONULATOL: ORIGIN OF THE 2',3',4'-OXYGENATION PATTERN

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Key Word Index—*Colutea arborescens*; Leguminosae; isoflavan; isoflavonoid; isomucronulatol; biosynthesis; phytoalexin.

Abstract—Feeding experiments with ^{14}C-labelled isoflavones in seedlings and pods of bladder senna (*Colutea arborescens*) have demonstrated that 7-hydroxy-4'-methoxyisoflavone (formononetin), 7,3'-dihydroxy-4'-methoxyisoflavone (calycosin), 7,2',3'-trihydroxy-4'-methoxyisoflavone (koparin) and 7,2'-dihydroxy-3',4'-dimethoxyisoflavone are excellent precursors of (3*R*)-isomucronulatol (7,2'-dihydroxy-3',4'-dimethoxyisoflavan). 7,2'-Dihydroxy-4'-methoxyisoflavone (2'-hydroxyformononetin) and 7-hydroxy-3',4'-dimethoxyisoflavone (cladrin) were, however, poor substrates. Thus, the biosynthetic sequence to isomucronulatol from formononetin involves 3'-hydroxylation, 2'-hydroxylation and then 3'-*O*-methylation, followed presumably by stereospecific reduction of 7,2'-dihydroxy-3',4'-dimethoxyisoflavone. Treatment of 2',3',4'-trimethoxyisoflavones with aluminium chloride in acetonitrile gives modest yields of 2',3'-dihydroxy derivatives rather than 2'-monohydroxyisoflavones, and thus provides a convenient access to 2',3'-dihydroxyisoflavones and related pterocarpans.

INTRODUCTION

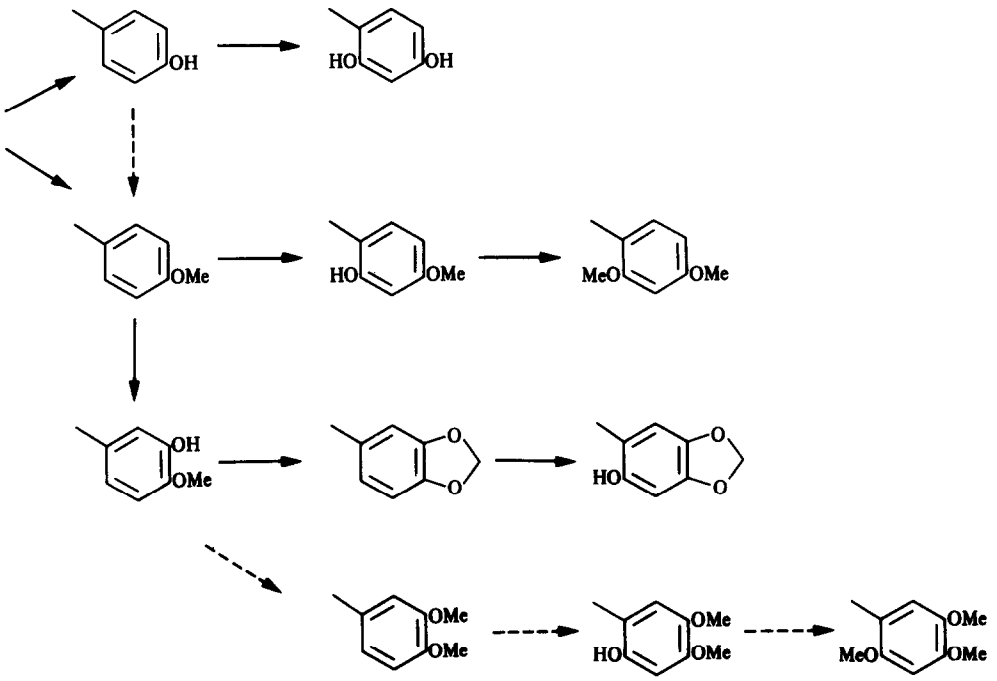
The huge variety of naturally occurring isoflavonoid structures now characterized [1, 2] arises because of changes in the oxidation level of the heterocyclic ring, formation of other ring systems, the introduction of alkyl substituents (especially hemiterpenoid), and particularly the oxygenation pattern on the aromatic rings. More complex oxygenation patterns are now appreciated to be developed only after formation of the basic isoflavonoid skeleton [1]. So far, only two chalcones, 2',4,4'-trihydroxychalcone (**1**) and 2',4,4',6'-tetrahydroxychalcone (**2**), have been demonstrated to act as isoflavonoid precursors, giving four primary isoflavones, daidzein (**3**), genistein (**5**) and their 4'-methyl ethers formononetin (**4**) and biochanin A (**6**). The latter two isoflavones appear to arise by a process involving methylation during the aryl migration sequence [1], rather than by direct methylation of the hydroxyisoflavones, although such a reaction can also occur [3]. A number of sequences leading to more complex oxygenations have been characterized by feeding experiments. Thus, in the acetate–malonate derived A-ring, the 7-hydroxy substitution of formononetin may be modified to give the 6,7-dihydroxyisoflavone texasin and then the 7-hydroxy-6-methoxy derivative afrormosin [4]. More information is available, however, for modifications in the shikimate-derived B-ring (Scheme 1). Thus, *meta*-hydroxylation of both 4'-hydroxy- and 4'-methoxyisoflavones has been demonstrated [5, 6], which, in the latter case, may be followed by further alkylation [7]. In the sequence leading to the 2'-hydroxy-4',5'-methylenedioxy pattern for maackiain [8] and pisatin [9], *ortho*-hydroxylation precedes *meta*, and 3',4'(≡ 4',5') disubstituted intermediates are involved. The formation of the methylenedioxy group precedes the *meta*-oxygenation, however, and in the case of pisatin, *O*-methylation in the A-ring occurs only after the B-ring pattern is completed [9]. The related 2',4',5'-trimethoxyisoflavones are intermediates in the biosynthesis of rotenoids [10] and although no evidence is available, probably arise by a similar sequence from the demonstrated 4'-methoxy precursor. In all the examples studied, any *C*-prenylation occurs after the final oxygenation pattern has been produced [10–12].

To extend our knowledge about the origins of isoflavonoid oxygenation patterns, we have studied the biosynthesis of the isoflavan (3*R*)-isomucronulatol (**7**) in seedlings and pods of bladder senna, *Colutea arborescens*. Isomucronulatol was first identified as a phytoalexin in *Glycyrrhiza glabra* leaves [13], and then as a constituent of *Gliricidia sepium* heartwood [14]. It has more recently been reported to function as a phytoalexin in *C. arborescens*, as well as in species of *Astragalus* and *Carmichaelia* [2]. This compound, 7,2'-dihydroxy-3',4'-dimethoxyisoflavan, possesses the uninvestigated 2',3',4' B-ring oxygenation pattern.

RESULTS AND DISCUSSION

Biosynthetic experiments

To provide a convenient, non-seasonal plant system, the formation of isomucronulatol in young, light-grown seedlings was investigated (Table 1). Seedlings were grown in moist vermiculite, then carefully uprooted and exposed to UV light (254 nm) or aqueous $CuCl_2$ solution (3×10^{-3} M), or a combination of these treatments to induce phytoalexin synthesis [6, 15]. After appropriate growing-on periods, plant material was extracted and isomucronulatol separated and quantified, using synthetic material as reference. In all cases, acceptable levels of isomucronulatol were observed, but there was little



Scheme 1. Demonstrated modifications in the B-ring of isoflavonoids.

Table 1. Isomucronulatol content of *Colutea arborescens* seedlings after various induction procedures

Age of seedlings (days)	No. of seedlings used	Induction method*	Induction period (hr)	Growing-on period (hr)	Isomucronulatol produced (mg)
21	15	None	None	None	0.21
21	15	$CuCl_2$	24	24	0.27
21	15	UV + $CuCl_2$	0.5/24	24	0.20
21	15	UV	0.5	24	0.18
14	15	UV + $CuCl_2$	0.5/24	24	0.11
28	20	$CuCl_2$	24	24	0.30
28	20	UV	0.5	24	0.26
28	20	None	None	None	0.23

*Aqueous $CuCl_2$ (3×10^{-3} M) was applied to roots; UV irradiation (254 nm) 5 cm from seedlings.

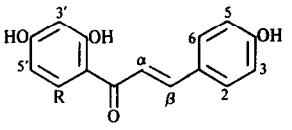
evidence to suggest that the induction treatment was producing any significant increase in isoflavan content. The bulk of the material was present without any additional stress treatment, and under these conditions therefore, isomucronulatol cannot be regarded as a phytoalexin/stress metabolite. In a preliminary feeding experiment, [Me-^{14}C]formononetin as its sodium salt was tested as a precursor in untreated seedlings and also in $CuCl_2$/UV-treated seedlings. After growing-on, the seedlings (*ca* 30) were worked up, and isomucronulatol was isolated, quantified and diluted with synthetic carrier, prior to acetylation and rigorous purification. Results were as follows:

(i) No treatment, 48 hr feeding period; 0.140 mg isomucronulatol, incorporation 0.38%, dilution 132.

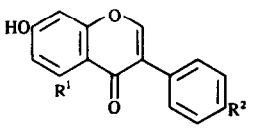
(ii) $CuCl_2$ treatment 24 hr, UV 0.5 hr, 24 hr feeding period; 0.147 mg isomucronulatol, incorporation 0.14%, dilution 308.

The better incorporation data observed in the untreated seedlings, experiment (i), led to this type of system being adopted for feeding experiments, and no induction processes were carried out.

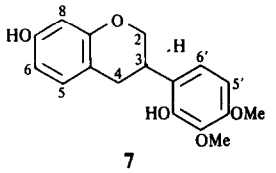
Logically, the elaboration of the 2'-hydroxy-3',4'-dimethoxy B-ring pattern from the 4'-methoxy of formononetin may proceed by any of the sequences indicated in Scheme 2. The involvement of such compounds was therefore tested by feeding the appropriate ^{14}C-labelled 7-hydroxyisoflavone and measuring incorporation data. This approach is limited in that no unique pathway may be operative, and that elaboration of the B-ring may not



R
1 H
2 OH



3 R^1 = H, R^2 = OH
4 R^1 = H, R^2 = OMe
5 R^1 = R^2 = OH
6 R^1 = OH, R^2 = OMe



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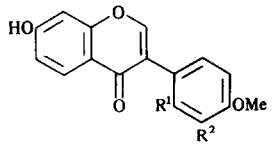
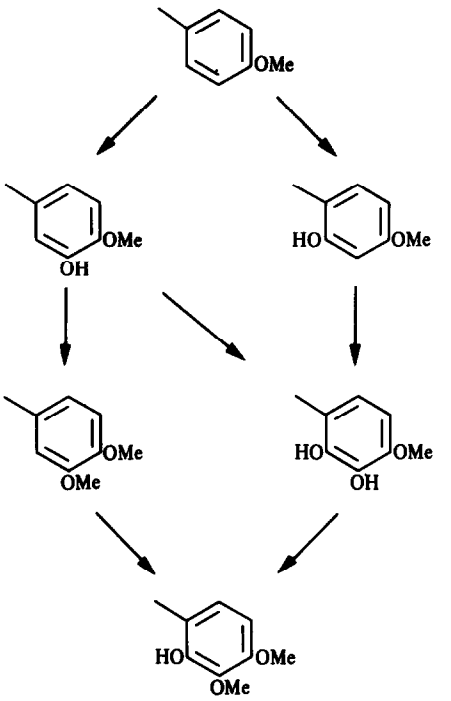


	R^1	R^2	
8	OH	H	2'-hydroxyformononetin
9	H	OH	calycosin
10	H	OMe	cladrin
11	OH	OH	koparin
12	OH	OMe	
13	OMe	OMe	

necessarily occur at the isoflavone oxidation level. Thus, 7-hydroxy-2',4'-dimethoxyisoflavone is not a precursor of sativan (7-hydroxy-2',4'-dimethoxyisoflavan), but vestitol (7,2'-dihydroxy-4'-methoxyisoflavan) is [7]. The results of the experiments are given in Table 2.



Scheme 2. Possible biosynthetic pathways for elaboration of the B-ring of isomucronulatol.

In the first series of experiments (A), the incorporation of ^{14}C-labelled formononetin was compared with those of the two isoflavones likely to be next in sequence, either 7,2'-dihydroxy-4'-methoxyisoflavone (2'-hydroxyformononetin) (**8**) or 7,3'-dihydroxy-4'-methoxyisoflavone (calycosin) (**9**). In the event, both were inferior to formononetin as precursors, although calycosin appeared the better of the two. The incorporation of calycosin was,

Table 2. Incorporation of ^{14}C-labelled isoflavones into isomucronulatol in *Colutea arborescens* seedlings and pods

Experiment*	Isoflavone fed	% Incorporation	Dilution
A (S)	Formononetin (**4**)†	0.260	245
	Calycosin (**9**)‡	0.082	791
	2'-Hydroxyformononetin (**8**)§	0.006	1.24×10^4
B (P)	Formononetin†	6.9	38
	Calycosin‡	5.1	72
	2'-Hydroxyformononetin§	0.12	2750
	Koparin (**11**)†	0.30	999
	7,2'-Dihydroxy-3',4'-dimethoxyisoflavone (**12**)†	6.1	71
C (S)	Cladrin (**10**)‡	0.01	9040
	Koparin†	0.013	7210
	7,2'-Dihydroxy-3',4'-dimethoxyisoflavone†	3.0	29
D (P)	Formononetin§	1.7	111
	Cladrin‡	0.13	2340
	Koparin†	1.05	70

*S, seedlings; P, pods.
†[2-^{14}C].
‡[*carbonyl*-^{14}C].
§[Me-^{14}C].

however, sufficiently large to suggest it might be a precursor of isomucronulatol and the depressed incorporation relative to formononetin could be the result of permeability/transport problems in the feeding experiment. Such problems have been minimized in other leguminous plant systems by injecting precursors into pod cavities [9, 12] and extremely high incorporations have often resulted.

The seed pods of *C. arborescens* are quite large, inflated structures with thin walls which become papery when ripe. Extraction of unripe pods showed that these also contain quite significant amounts of isomucronulatol, levels of which did not noticeably change after injection of aqueous $CuCl_2$ into the pod. Larger scale extraction of unripe pods (50 g) resulted in the isolation of crystalline isomucronulatol (6.3 mg), sufficient for optical rotation measurements. An $[\alpha]_D$ (acetone) of $-13.7°$ was observed, comparing well with that reported for (−)-(3*R*)-isomucronulatol from *Gliricidium sepium*, viz. $-5.3°$ (acetone) [14]. Thus *Colutea* isomucronulatol can also be assigned the 3*R* configuration.

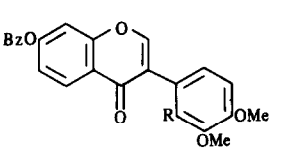
Feeding experiments with pods were also carried out without any form of induction, precursor solutions of the isoflavones (as sodium salts) being injected into the detached pods (6–7), which were then kept under humid conditions for the 48 hr feeding period. In this series of experiments (B), ^{14}C-labelled formononetin and calycosin were very efficient precursors, 6.9 and 5.1% incorporations, respectively. In marked contrast, 2′-hydroxyformononetin was a relatively poor precursor. Improved absorption in pod tissue had thus enabled the two dihydroxyisoflavones to be distinguished with respect to precursor efficiency. Of the other isoflavones fed in this series of experiments, that with the isomucronulatol substitution pattern, 7,2′-dihydroxy-3′,4′-dimethoxyisoflavone (**12**), was also an efficient precursor (6.1% incorporation), but 7,2′,3′-trihydroxy-4′-methoxyisoflavone (koparin) (**11**) was incorporated only to the extent of 0.3%. With the improved incorporation of calycosin, it now remained to ascertain the role of koparin or 7-hydroxy-3′,4′-dimethoxyisoflavone (cladrin) (**10**) as precursors. The figure already achieved with koparin, however, made cladrin, perhaps, more likely.

The experiments (C) were carried out with seedlings, since seasonal problems meant pod tissue was unobtainable at this time. Again, 7,2′-dihydroxy-3′,4′-dimethoxyisoflavone was well incorporated, suggesting there were few transport/absorption problems associated with this isoflavone, and that the substitution pattern was most probably built up at the isoflavone level, with reduction to the isoflavan being a late stage in the biosynthesis. Cladrin and koparin were, however, both very poorly incorporated, though logically one or the other should be a precursor. In this case, the solution was found by employing a different feeding technique. Although most phenolic isoflavones can easily be solubilized by dissolving them in sodium hydroxide and then adding buffer, the alkaline conditions initially employed could well affect compounds containing catechol groupings by promoting oxidation reactions. To avoid this, experiments (D) utilized 2-methoxyethanol–Tween–water solutions useful for non-phenolic substrates [9]. Feedings were also delayed until pod tissue again became available, and this time a marked difference was observed between the incorporations of koparin (1.05%) and cladrin (0.13%), cf. formononetin (1.7%).

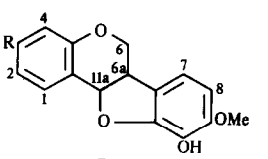
The results allow the pathway from formononetin to be formulated as: formononetin → calycosin → koparin → 7,2′-dihydroxy-3′,4′-dimethoxyisoflavone, with subsequent stereospecific reduction sequences to give the (3*R*)-isoflavan. By analogy with other systems [6, 7, 15], it is probable that 7,2′-dihydroxy-3′,4′-dimethoxy-isoflavanone and -isoflavanol are involved. The possibility that a metabolic grid may be operative is not excluded, but the convincing incorporations of the labelled isoflavones suggest it is highly likely that the substitution pattern of isomucronulatol is elaborated at the isoflavone oxidation level.

Synthetic studies

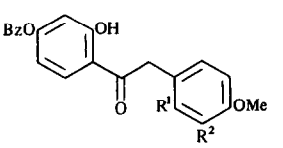
(±)-Isomucronulatol was synthesized by catalytic hydrogenation of 7,2′-dibenzyloxy-3′,4′-dimethoxyisoflavone (**14**), prepared by standard thallium nitrate oxidation of the appropriate chalcone acetate [16]. Spectral data for the synthetic compound were identical to those reported for the natural product [14]. Initially, an alternative route exploiting the specific demethylation of 2′-methoxyisoflavones by $AlCl_3$ in MeCN [17] was devised. Treatment of 7-benzyloxy-2′,3′,4′-trimethoxyisoflavone (**15**) with $AlCl_3$–MeCN (reflux, overnight) gave not the expected 7,2′-dihydroxy-3′,4′-dimethoxyisoflavone (**12**), but 7,2′,3′-trihydroxy-4′-methoxyisoflavone (**11**) (koparin) in 25–30% yield. By reducing reaction times to 1.5–2 hr, it was found possible to alter the composition of the product mixture, giving the required isoflavone (**12**) (15%) and 7-hydroxy-2′,3′,4′-trimethoxyisoflavone (**13**) (15%) together with unreacted starting material. Although this is not a useful route for the synthesis of **12** and hence isomucronulatol, the $AlCl_3$ demethylation of **15** to koparin does give a fairly efficient access to 2′,3′-dihydroxy isoflavones, avoiding the necessity of protecting these positions by benzylation or methyleneation [18] at the beginning of the synthetic sequence. 7,2′,3′,4′-Tetramethoxyisoflavone was similarly converted into 2′,3′-dihydroxy-7,4′-dimethoxyisoflavone in comparable yield. In addition, $AlCl_3$–MeCN treatment of 7,2′-dibenzyloxy-3′,4′-dimethoxyisoflavone (**14**) also yielded koparin. Thus, it appears likely that 2′-demethylation is the initial step in the reaction (after debenzylation, if appropriate), and that there is then a second selective demethylation, perhaps as a consequence of the combined effects



R
14 OBz
15 OMe



R
16 OH
17 OMe



18 $R^1 = R^2 = H$
19 $R^1 = OBz$, $R^2 = OMe$

of the carbonyl and 2'-hydroxyl chelating with the $AlCl_3$. 2',3'-Didemethylation of 7-hydroxy-2',3',4'-trimethoxyisoflavone (**13**) to koparin using $AlCl_3$ in nitrobenzene has, in fact, been reported previously [19], and in rather better yields than described here. The significance of the reaction and its usefulness were, however, not commented upon. The role of the solvent has not been investigated further; in the 2'-demethylations studied [17] only acetonitrile was effective for selective dealkylation.

Treatment of koparin with sodium borohydride efficiently yielded vesticarpan (**16**), which was selectively methylated to methylnissolin (**17**). Thus, ready access to 2',3'-dihydroxyisoflavones via the $AlCl_3$ demethylation gives further access to pterocarpans with the 3,9,10-substitution pattern.

The $AlCl_3$–MeCN demethylation was utilized further in the synthesis of labelled substrates. Thus, unlabelled 7,2'-dibenzyloxy-3',4'-dimethoxyisoflavone (**14**) was converted into the deoxybenzoin (**19**) by alkaline hydrolysis, and [2-^{14}C]isoflavone **14** was produced by reacting **19** with triethyl [^{14}C]orthoformate in pyridine–piperidine [20]. Catalytic transfer debenzylation yielded [2-^{14}C]-7,2'-dihydroxy-3',4'-dimethoxyisoflavone (**12**) whereas $AlCl_3$–MeCN dealkylation gave [2-^{14}C]koparin (**11**). The synthesis of 2-^{14}C-labelled isoflavones via triethyl [^{14}C]orthoformate was also exploited for the preparation of [2-^{14}C]formononetin from deoxybenzoin (**18**). This represents an extremely useful and uncomplicated route to labelled isoflavones, but for success requires an excess (3–4 mol) of the labelled orthoformate in the reaction. Thus, the synthesis is inefficient with respect to radiochemical, which is not easily recovered. However, this disadvantage is easily outweighed by the length of alternative syntheses, such as we have employed earlier for the preparation of [*carbonyl*-^{14}C]calycosin and in the present work for [*carbonyl*-^{14}C]cladrin. These involved standard sequences from [*carbonyl*-^{14}C]resacetophenone via chalcones and thallium nitrate oxidation.

EXPERIMENTAL

TLC. TLC was carried out using 0.5 mm layers of silica gel (Merck Kieselgel $60GF_{254}$) and TLC zones were eluted with Me_2CO (Analar).

Radiochemicals. Triethyl [^{14}C]orthoformate (59 mCi/mM) was purchased from Amersham. [Me-^{14}C]Formononetin (0.568 mCi/mM) [15], [Me-^{14}C]-2'-hydroxyformononetin (0.518 mCi/mM) [6] and [*carbonyl*-^{14}C]calycosin (0.0271 mCi/mM) [8] were available from earlier experiments, or were synthesized according to published procedures.

Isomucronulatol production in seedlings. Seeds of *Colutea arborescens* (Chiltern Seeds Ltd., Ulverston, 10 g) were scarified by scratching the seed coat with a scalpel, then surface-sterilized in 2% NaOCl soln (15 min), EtOH (10 min), and finally washed with H_2O. The seeds were sown in trays containing moist vermiculite, and germinated in a growth cabinet at 25° in a 12 hr light (4000 lx)–12 hr dark cycle for 28 days, H_2O being added as necessary. The seedlings were carefully uprooted and batches of 30 seedlings were placed in 25 ml beakers. Labelled phenolic precursors (*ca* 0.5 mg) were applied to the roots as their Na salts, by dissolving them in the minimum amount of aq. NaOH (0.5 M) then adding phosphate buffer (0.1 M, pH 7.0, 2 ml) and H_2O (4 ml). The seedlings were returned to the growth cabinet for 48 hr. After the feeding period, the seedlings were homogenized in a mortar with ground glass and a little H_2O. The slurry and the feeding soln were then poured into hot EtOH (200 ml), cooled, filtered, and the solids re-extracted with hot EtOH (3 × 200 ml). The combined extracts were evapd to dryness, H_2O (50 ml) was added, then were extracted with Et_2O (100 ml, then 4 × 50 ml). The extracts were bulked, evapd and separated by TLC (hexane–Me_2CO, 2:1), using isomucronulatol as reference marker. Isomucronulatol was eluted, purified further by TLC (hexane–EtOAc, 3:2) and quantified by UV absorption (EtOH), λ_{max} 280 nm, log ε 3.66. The compound was diluted with synthetic (±)-carrier (*ca* 20 mg) and acetylated in dry pyridine (5 ml) with Ac_2O (0.5 ml) at room temp. overnight. Usual work-up followed by TLC (hexane–Me_2CO, 2:1) gave (±)-isomucronulatol diacetate, mp 122–124°, which was purified to constant sp. act. from aq. MeOH.

Isomucronulatol production in pods. Feeding experiments in *C. arborescens* pods (collected from mature bush on the University of Nottingham campus) were performed during August and September. Labelled phenolic precursors (*ca* 0.4 mg) were administered in aq. NaOH (0.5 M), Pi buffer (10 ml) and H_2O (20 ml) as before, injecting the soln into the pods (6–7) using a hypodermic syringe. The pods were manipulated to coat the inside surface with the soln, then placed on moist filter paper in the growth cabinet for 48 hr under the conditions used for seedlings. The pods, together with residual feeding solns, were extracted as described for seedlings. Isomucronulatol, after UV quantification, was further purified by TLC (hexane–Me_2CO, 3:2; hexane–EtOAc–MeOH, 6:4:1; $CHCl_3$–Me_2CO, 10:1). A sample was then quantified by UV absorption and counted directly without acetylation.

In later experiments, the feeding soln was modified by dissolving the precursor in 2-methoxyethanol (3 ml), then adding Tween 20 (6 drops) and H_2O (25 ml).

(−)-(3R)-*Isomucronulatol.* Large-scale extraction of *C. arborescens* pods (50 g) yielded isomucronulatol (6.3 mg), recrystallized from aq. MeOH, mp 148–150° (lit. [14] 152–153°); $[\alpha]_D^{20}$ −19.4° (EtOH; *c* 6.24 mg/ml), −13.7° (Me_2CO; *c* 6.20 mg/ml), lit. [14] $[\alpha]_D^{22}$ −5.3° (Me_2CO); UV λ_{max}^{EtOH} nm (log ε): 280 (3.66), 289; EIMS *m/z* (rel. int.): 302 $[M]^+$ (60), 180 (100), 167 (40), 151 (13), 133 (15), 123 (11); ^{1}H NMR (250 MHz, $(CD_3)_2CO$, TMS): δ 8.15 (1H, *s*, OH), 7.98 (1H, *s*, OH), 6.90 (1H, *d*, *J* = 8.2 Hz, H-5), 6.84 (1H, *d*, *J* = 8.6 Hz, H-6'), 6.51 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.37 (1H, *dd*, *J* = 8.2, 2.4 Hz, H-6), 6.28 (1H, *d*, *J* = 2.4 Hz, H-8), 4.25 (1H, *ddd*, *J* = 10.2, 3.4, 1.9 Hz, H-2_R), 3.99 (1H, *t*, *J* = 10.1 Hz, H-2_S), 3.82 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 3.46 (1H, *m*, H-3), 2.97 (1H, *dd*, *J* = 15.5, 10.2 Hz, H-4_R), 2.82 (1H, *ddd*, *J* = 15.5, 5.4, 1.3 Hz, H-4_S).

2,4'-*Dibenzyloxy*-3,4-*dimethoxy*-2'-*hydroxychalcone.* 2-Hydroxy-3,4-dimethoxybenzaldehyde (2 g) was stirred and heated at 60° with dry K_2CO_3 (2 g), KI (0.3 g) and BzCl (1.67 g) in dry DMF (15 ml) for 1.5 hr. The mixture was poured into H_2O, extracted with EtOAc (3 × 100 ml) and the extracts were washed with H_2O. The extracts were then evapd to yield 2-benzyloxy-3,4-dimethoxybenzaldehyde (2.6 g) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$, TMS): δ 10.02 (1H, *s*, CHO), 7.46 (1H, *d*, *J* = 9 Hz, H-6), 7.31 (5H, *s*, $-CH_2\underline{Ph}$), 6.64 (1H, *d*, *J* = 9 Hz, H-5), 5.20 (2H, *s*, $-C\underline{H}_2Ph$), 3.92 (6H, *s*, OMe × 2).

Without further purification, this aldehyde (2.5 g) and 4'-benzyloxy-2'-hydroxyacetophenone (2.3 g) were dissolved in warm EtOH (100 ml). KOH (25 g) in H_2O (25 ml) was added and the solution stirred at room temp. overnight. The mixture was poured into ice-H_2O, acidified with conc. HCl, and the ppt. filtered off, washed with H_2O and air-dried. The chalcone was recrystallized from $CHCl_3$–MeOH, yield 3.4 g, mp 132–135° (lit. [16] 136–137°). ^{1}H NMR (60 MHz, $CDCl_3$, TMS): δ 7.90 (1H, *d*, *J* = 14 Hz, H-β), 7.72 (1H, *d*, *J* = 9 Hz, H-6'), 7.34 (10H, *s*, $-CH_2\underline{Ph}$ × 2), 7.28 (1H, *d*, *J* = 14 Hz, H-α), 6.67 (1H, *d*, *J* = 9 Hz,

H-6), 6.38 (3H, *m*, H-3', H-5', H-5), 5.09 (2H, *s*, $-C\underline{H}_2Ph$), 5.03 (2H, *s*, $-C\underline{H}_2Ph$), 3.88 (6H, *s*, OMe × 2).

7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone (**14**). The above chalcone (1.2 g) was acetylated with Ac_2O (2.5 ml) in dry pyridine (25 ml) at room temp. overnight. The chalcone acetate was isolated as usual, then, without purification, dissolved in MeOH (250 ml) and stirred with $Tl(NO_3)_3 \cdot 3H_2O$ (1.55 g) at room temp. overnight. Solid KOH (3.1 g) was added, and the mixture stirred for a further 1 hr. After neutralization with conc. HCl, 10% HCl (25 ml) was added, and the mixture heated under reflux for 2 hr, then filtered hot. The filtrate was concd, poured into H_2O, and extracted with EtOAc (3 × 100 ml). The combined extracts were washed with H_2O, evapd, and the residue was crystallized from MeOH to afford the isoflavone (0.9 g), mp 84–95° (lit. [16] 126–128°). Despite this discrepancy in mps, the spectral data were satisfactory. ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 8.15 (1H, *d*, *J* = 9 Hz, H-5), 7.66 (1H, *s*, H-2), 7.41 (5H, *s*, $-CH_2\underline{Ph}$), 7.15 (5H, *s*, $-CH_2\underline{Ph}$), 6.94 (3H, *m*, H-6, H-8, H-6'), 6.73 (1H, *d*, *J* = 9 Hz, H-5'), 5.15 (2H, *s*, $-C\underline{H}_2Ph$), 5.04 (2H, *s*, $-C\underline{H}_2Ph$), 3.96 (3H, *s*, OMe), 3.90 (3H, *s*, OMe).

(±)-*Isomucronulatol*. 7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone (250 mg) was hydrogenated in glacial HOAc (35 ml) over Pd/C catalyst (10%, 200 mg) overnight at room temp. The mixture was filtered, evapd and the residue recrystallized from MeOH to give (±)-isomucronulatol (180 mg), mp 186–189° (lit. [16] 190–192°), IR, UV, MS, ^1H NMR as for the natural compound.

4'-Benzyloxy-2'-hydroxy-2,3,4-trimethoxychalcone. 4'-Benzyloxy-2'-hydroxyacetophenone (2 g) in EtOH (80 ml) was stirred at room temp. overnight with 2,3,4-trimethoxybenzaldehyde (1.7 g) and KOH (20 g) in H_2O (20 ml). The chalcone was isolated as above, and recrystallized from $CHCl_3$–MeOH, yield 2.4 g, mp 135–137°. ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 8.01 (1H, *d*, *J* = 14 Hz, H-β), 7.75 (1H, *d*, *J* = 9 Hz, H-6'), 7.50 (1H, *d*, *J* = 14 Hz, H-α), 7.33 (5H, *s*, $-CH_2\underline{Ph}$), 7.28 (1H, *d*, *J* = 9 Hz, H-6), 6.64 (1H, *d*, *J* = 9 Hz, H-5), 6.45 (2H, *m*, H-3',H-5'), 5.06 (2H, *s*, $-C\underline{H}_2Ph$), 3.95 (3H, *s*, OMe), 3.88 (6H, *s*, OMe × 2).

7-Benzyloxy-2',3',4'-trimethoxyisoflavone (**15**). The above chalcone (1.5 g) was acetylated, and the crude acetate dissolved in MeOH (340 ml) and stirred at room temp. overnight with $Tl(NO_3)_3 \cdot 3H_2O$ (1.9 g). Following work-up as described above, the isoflavone was recrystallized from aq. MeOH, yield 1.2 g, mp 162–167°. ^1H NMR (60 MHz, $CDCl_3$, TMS): δ8.27 (1H, *d*, *J* = 9 Hz, H-5), 7.96 (1H, *s*, H-2), 7.49 (5H, *s*, $-CH_2\underline{Ph}$), 7.12 (3H, *m*, H-6, H-8, H-6'), 6.76 (1H, *d*, *J* = 9 Hz, H-5'), 5.21 (2H, *s*, $-C\underline{H}_2Ph$), 3.97 (3H, *s*, OMe), 3.91 (3H, *s*, OMe), 3.85 (3H, *s*, OMe).

Reaction of **15** with $AlCl_3$/MeCN. (i) 7-Benzyloxy-2',3',4'-trimethoxyisoflavone (0.5 g) was heated under reflux with $AlCl_3$ (2.5 g) in dry MeCN (25 ml) overnight. The soln was concd, and cold 10% HCl slowly added. The mixture was extracted with EtOAc (3 × 50 ml) and the extracts were washed with H_2O. The evapd residue was recrystallized from MeOH to give 7,2',3'-trihydroxy-4'-methoxyisoflavone (koparin) (**11**), 100 mg, mp 267–270°, (lit. [18] 269–271°): UV λ_{max}^{EtOH} nm (log ε): 247 (4.40), 264, 305; ^1H NMR (60 MHz, $(CD_3)_2CO$–$(CD_3)_2SO$, TMS): δ8.34 (1H, *s*, H-2), 8.08 (1H, *d*, *J* = 9 Hz, H-5), 7.18 (1H, *dd*, *J* = 9, 2 Hz, H-6), 7.05 (1H, *d*, *J* = 2 Hz, H-8), 6.82 (1H, *d*, *J* = 9 Hz, H-6'), 6.58 (1H, *d*, *J* = 9 Hz, H-5'), 3.84 (3H, *s*, OMe).

(ii) 7-Benzyloxy-2',3',4'-trimethoxyisoflavone (180 mg) in dry MeCN (10 ml) was heated under reflux for 1.5 hr with $AlCl_3$ (0.9 g). The mixture was worked up as above, and the residue separated by TLC ($CHCl_3$–MeOH, 25:1). Two major products, in addition to unreacted starting material, were isolated and crystallized from aq. MeOH. 7,2'-Dihydroxy-3',4'-dimethoxyisoflavone (**12**) (20 mg), mp 240–243°; UV λ_{max}^{EtOH} nm (log ε): 247 (4.37), 260, 306; ^1H NMR (60 MHz, $(CD_3)_2CO$–$(CD_3)_2SO$, TMS): δ 8.31 (1H, *s*, H-2), 8.07 (1H, *d*, *J* = 9 Hz, H-5), 7.09 (3H, *m*, H-6, H-8, H-6'), 6.84 (1H, *d*, *J* = 9 Hz, H-5'), 3.94 (3H, *s*, OMe), 3.85 (3H, *s*, OMe). 7-Hydroxy-2',3',4'-trimethoxyisoflavone (**13**) (20 mg), mp 253–256°; UV λ_{max}^{EtOH} nm: 247, 255, 296, 304; ^1H NMR (60 MHz, $(CD_3)_2CO$–$(CD_3)_2SO$, TMS): δ 8.15 (1H, *s*, H-2), 8.08 (1H, *d*, *J* = 9 Hz, H-5), 7.10 (4H, *m*, H-6, H-8, H-6', H-5'), 3.95 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 3.81 (3H, *s*, OMe).

2-Benzyloxy-3,4-dimethoxybenzyl-4-benzyloxy-2-hydroxyphenylketone (**19**). 7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone (160 mg) was dissolved in EtOH (70 ml) and H_2O (35 ml), KOH (6 g) was added, then the mixture was stirred under reflux for 1 hr. The soln was cooled, concd, acidified with 10% HCl and extracted with EtOAc (3 × 50 ml). The extracts were washed with H_2O, evapd and purified by TLC (hexane–Me_2CO, 2:1) to yield the deoxybenzoin (130 mg) as a solid, mp 74–78°; ^1H NMR (60 MHz, $(CD_3)_2CO$, TMS): δ 7.88 (1H, *d*, *J* = 9 Hz, H-6), 7.50 (5H, *s*, $-CH_2\underline{Ph}$), 7.39 (5H, *s*, $-CH_2\underline{Ph}$), 6.95 (1H, *d*, *J* = 9 Hz, H-6'), 6.86 (1H, *d*, *J* = 9 Hz, H-5'), 6.58 (2H, *m*, H-3, H-5), 5.27 (2H, *s*, $-CH_2Ph$), 5.13 (2H, *s*, $-CH_2Ph$), 4.25 (2H, *s*, $COC\underline{H}_2Ar$), 3.84 (6H, *s*, OMe × 2).

[2-^{14}C]-7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone. The above deoxybenzoin (55 mg) was dissolved in dry pyridine (0.6 ml) and dry piperidine (20 μl). Triethyl orthoformate (70 μl) and triethyl [^{14}C]orthoformate (100 μl, 50 μCi) were added and the mixture was stirred and heated in a Reactivial at 110° for 4 days. The soln was pipetted into ice-cold H_2O (50 ml), acidified with conc. HCl and extracted with EtOAc (3 × 50 ml). The bulked extracts were washed with H_2O and evapd. The residue was separated by TLC (hexane–EtOAc, 3:2) to give the labelled isoflavone (36 mg).

[2-^{14}C]-7,2'-Dihydroxy-3',4'-dimethoxyisoflavone. [2-^{14}C]-7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone (15 mg) was debenzylated by stirring and heating under reflux with cyclohexene (2 ml) and $Pd(OH)_2$–C catalyst (20%, 25 mg) in EtOH (4 ml) for 1.5 hr. The mixture was evapd and the residue purified to constant sp. act. by TLC ($CHCl_3$–MeOH, 20:1; $CHCl_3$–*i*-PrOH, 10:1) to yield the required isoflavone, 7.6 mg, sp. act. 0.140 mCi/mM.

[2-^{14}C]-7,2',3'-Trihydroxy-4'-methoxyisoflavone (koparin). [2-^{14}C]-7,2'-Dibenzyloxy-3',4'-dimethoxyisoflavone (15 mg) was heated under reflux overnight with $AlCl_3$ (80 mg) in dry MeCN (5 ml). After cooling, the soln was concd and cold 10% HCl added. The mixture was extracted with EtOAc (3 × 25 ml), and the extracts were washed with H_2O and evapd. [2-^{14}C]Koparin (1.8 mg) was isolated and purified to constant sp. act. (0.140 mCi/mM) by TLC (toluene–HCO_2Et–HCO_2H, 5:4:1; $CHCl_3$–MeOH, 9:1; $CHCl_3$–*i*-PrOH, 10:1).

[2-^{14}C]Formononetin. 4-Methoxybenzyl-2,4-dihydroxyphenylketone (12 mg) was stirred and heated at 110° in a Reactivial for 3 days with dry pyridine (1.5 ml), dry piperidine (10 μl), triethyl orthoformate (20 μl) and triethyl [^{14}C]orthoformate (75 μl, 37.5 μCi). The mixture was worked up as above, and [2-^{14}C]formononetin (7.2 mg) isolated by TLC (hexane–Me_2CO, 2:1) and purified to constant sp. act. (0.192 mCi/mM) by further TLC ($CHCl_3$–MeOH, 25:1; $CHCl_3$–*i*-PrOH, 10:1; hexane–EtOAc–MeOH, 6:4:1).

[carbonyl-^{14}C]-4'-Benzyloxy-3,4-dimethoxy-2'-hydroxychalcone. [*carbonyl*-^{14}C]-4'-Benzyloxy-2'-hydroxyacetophenone [8] (0.281 mCi/mM, 17 mg) and 3,4-dimethoxybenzaldehyde (12 mg) in EtOH (2 ml) were treated with KOH (135 mg) in H_2O (0.13 ml), and the soln was stirred at room temp. overnight. The mixture was poured into H_2O, acidified with conc. HCl and extracted with EtOAc (3 × 20 ml). The extracts were washed with H_2O and evapd. The residue was separated by TLC (hexane–Me_2CO, 2.1; hexane–*i*-PrOH, 91:9) to give the chal-

cone (8 mg). Unlabelled material recrystallized from $CHCl_3$–MeOH had mp 140–143°, ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 7.96 (1H, *d*, J = 14 Hz, H-β), 7.88 (1H, *d*, J = 9 Hz, H-6'), 7.47 (1H, *d*, J = 14 Hz, H-α), 7.35 (5H, *s*, $-CH_2\underline{Ph}$), 7.12 (2H, *m*, H-2, H-6), 6.91 (1H, *d*, J = 9 Hz, H-5), 6.50 (2H, *m*, H-3',H-5'), 5.09 (2H, *s*, $-C\underline{H}_2Ph$), 3.98 (3H, *s*, OMe), 3 92 (3H, *s*, OMe).

[carbonyl-^{14}C]-7-*Benzyloxy*-3',4'-*dimethoxyisoflavone*. The above chalcone (8 mg) was acetylated in dry pyridine (5 ml) with Ac_2O (0.5 ml) at room temp. overnight. The crude acetate was dissolved in MeOH (3 ml) and stirred at room temp. overnight with $Tl(NO_3)_3 \cdot 3H_2O$ (20 mg). KOH (30 mg) was added, and the mixture stirred for a further 1 hr. After neutralization with conc. HCl, 10% HCl (2 ml) was added and the mixture heated under reflux for 2 hr The mixture was concd, diluted with H_2O and extracted with EtOAc (3 × 10 ml). The extracts were washed with H_2O, evapd, and the residue was purified by TLC (hexane–Me_2CO, 2:1) to yield the isoflavone. Unlabelled material recrystallized from MeOH had mp 147–149°; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 8.18 (1H, *d*, J = 9 Hz, H-5), 7 85 (1H, *s*, H-2), 7.37 (5H, *s*, $-CH_2\underline{Ph}$), 7.14 (2H, *m*, H-6, H-8), 6.90 (3H, *m*, H-2', H-5', H-6'), 5.11 (2H, *s*, $-C\underline{H}_2Ph$), 3.91 (3H, *s*, OMe), 3.86 (3H, *s*, OMe).

[carbonyl-^{14}C]-7-*Hydroxy*-3',4'-*dimethoxyisoflavone* (*cladrin*). The benzyloxyisoflavone from above was debenzylated by stirring and heating under reflux with cyclohexene (1 ml), $Pd(OH)_2$–C catalyst (20%, 5 mg) in EtOH (2 ml) for 1 hr. After evapn, the residue was separated by TLC ($CHCl_3$–MeOH, 9:1) to give the required hydroxyisoflavone (1.2 mg), which was purified to constant sp. act. (0.256 mCi/mM) by further TLC (hexane–EtOAc–MeOH, 6:4:1; $CHCl_3$–*i*-PrOH, 10:1; hexane–Me_2CO, 1:1). Unlabelled material recrystallized from MeOH had mp 260–262° (lit [21] 257–258°). UV λ_{max}^{EtOH} nm (log ε): 218, 248 (4.39), 257, 285, 307; ^1H NMR (60 MHz, $CDCl_3$–$(CD_3)_2SO$, TMS): δ 8.17 (1H, *s*, H-2), 8.05 (1H, *d*, J = 9 Hz, H-5), 7.00 (5H, *m*, H-6, H-8, H-2', H-5', H-6'), 3.87 (6H, *s*, OMe × 2).

2',3'-*Dihydroxy*-7,4'-*dimethoxyisoflavone*. 7,2',3',4'-Tetramethoxyisoflavone (150 mg) was heated under reflux overnight with $AlCl_3$ (0.8 g) in dry MeCN (10 ml). The soln was concd and treated with cold 10% HCl. The mixture was extracted with EtOAc (3 × 25 ml) and the extracts were washed with H_2O The evapd residue was separated by TLC ($CHCl_3$–*i*-PrOH, 10:1) and the major band eluted. 2',3'-Dihydroxy-7,4'-dimethoxyisoflavone (20 mg) was obtained by recrystallization from aq. MeOH, mp 205–209°, UV λ_{max}^{EtOH} nm: 245, 266, 302; ^1H NMR (60 MHz, $CDCl_3$–$(CD_3)_2SO$, TMS): δ 8.12 (1H, *s*, H-2), 8.05 (1H, *d*, J = 9 Hz, H-5), 7.06 (1H, *dd*, J = 9, 2.5 Hz, H-6), 6.97 (1H, *d*, J = 2.5 Hz, H-8), 6.75 (1H, *d*, J = 9 Hz, H-6'), 6.50 (1H, *d*, J = 9 Hz, H-5'), 3.98 (3H, *s*, OMe), 3 91 (3H, *s*, OMe).

(±)-3-*Hydroxy*-9,10-*dimethoxypterocarpan* (*methylnissolin*) (**17**). Koparin (40 mg) was dissolved in EtOH (Analar, 5 ml) and dry THF (5 ml). $NaBH_4$ (250 mg) was added slowly and the mixture stirred at room temp. for 24 hr. The mixture was concd, treated with 10% HCl (20 ml), and extracted with EtOAc (3 × 30 ml). The extracts were washed with H_2O and evapd. TLC (hexane–Me_2CO, 2:1) of the residue gave unreacted starting material and a second major band which was rechromatographed ($CHCl_3$–Me_2CO, 10:1). This compound had UV λ_{max}^{EtOH} nm: 275, 279, 284, identical to that reported for natural vesticarpan (3,10-dihydroxy-9-methoxypterocarpan) (**16**) [19]. Selective methylation (CH_2N_2) (Ingham, J. L., personal communication) gave (±)-methylnissolin, identical to natural material [22]. UV λ_{max}^{EtOH} nm: 274, 278, 284; ^1H NMR (250 MHz, $CDCl_3$, TMS): δ 7.45 (1H, *d*, J = 8.5 Hz, H-1), 6.89 (1H, *d*, J = 8.1 Hz, H-7), 6.57 (1H, *dd*, J = 8.5, 2.5 Hz, H-2), 6.47 (1H, *d*, J = 8.2 Hz, H-8), 6.41 (1H, *d*, J = 2.4 Hz, H-4), 5.53 (1H, *d*, J = 6.6 Hz, H-11a), 4.26 (1H, *dd*, J = 10.7, 4.6 Hz, H-6_{eq}), 3.93 (3H, *s*, OMe), 3.84 (3H, *s*, OMe), 3.66 (1H, *m*, H-6_{ax}), 3.57 (1H, *m*, H-6a).

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