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PHASEOLLIN FORMATION AND METABOLISM IN *PHASEOLUS VULGARIS**

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; French bean; isoflavanone; pterocarpan; isoflavan; phytoalexin.

Abstract—Following fungal-inoculation, *P. vulgaris* was found to produce small amounts of 7,4′-dihydroxyisoflavone (daidzein), 7,2′,4′-trihydroxyisoflavone, 7,2′,4′-trihydroxyisoflavanone, (6a*R*, 11a*R*)-3,9-dihydroxypterocarpan, and (3*R*)-7,2′,4′-trihydroxyisoflavan. The structures of the latter four compounds were confirmed by synthesis. The principal pterocarpans isolated were phaseollidin and phaseollin and ORD spectra indicate that these compounds have the same (6a*R*, 11a*R*)-configuration as 3,9-dihydroxypterocarpan. A pathway leading to phaseollidin and phaseollin is proposed involving 2′-hydroxylation of daidzein, reduction to the isoflavanone, further reduction, dehydration and cyclization to the pterocarpan, and prenylation to give phaseollidin and then cyclization and dehydrogenation to give phaseollin. No evidence of prenylation at the isoflavone or isoflavanone stage was obtained. The phaseollin metabolite, (6a*S*, 11a*S*)-6a-hydroxyphaseollin, was also detected.

INTRODUCTION

The pterocarpan phaseollin (**7**) was the first post-infectionally formed antifungal isoflavonoid identified from *Phaseolus vulgaris* [1]. The related pterocarpan phaseollidin (**6**) was subsequently identified from inoculation droplets [2]. These pterocarpans together with kievitone and phaseollinisoflavan (**10**) constitute the major antifungal isoflavonoids produced by *P. vulgaris* following fungal inoculation [3].

Phaseollin is believed to play a primary role in host plant defense [4]. An examination of the biosynthesis of phaseollin is necessary as part of the elucidation of the mechanism of resistance and experiments with labelled substrates have indicated that phaseollin is formed from cinnamic acid and acetate via the isoflavone daidzein (**1a**) [5]. However, little else is known about the isoflavonoid portion of the pathway. As several alternative routes may be postulated for phaseollin biosynthesis, a detailed examination of the isoflavonoids produced by *P. vulgaris* was undertaken. The isoflavonoids of *P. vulgaris* occur in two distinct classes: those with and those without an oxygen at C-5 (isoflavone system). The 5-hydroxylated isoflavonoids (e.g. kievitone) obtained from the extracts have been described previously [6, 7]. This paper reports the isolation and characterization of the 5-deoxyisoflavonoids **1a**, **2a**, **3a**, **5a**, and **9a** from *P. vulgaris* and a pathway leading to phaseollin is proposed and discussed.

RESULTS

Pod cavities of *P. vulgaris* were inoculated with a spore suspension of *Monilinia fructicola* (Wint.) Honey and incubated for 20 hr. Subsequent analysis of the inoculation fluid showed the presence of numerous 5-deoxy- and 5-hydroxy-isoflavonoids. The amounts of these compounds isolated and their behaviour on Si gel and polyamide TLC are shown in Table 1. Among the 5-deoxyisoflavonoids isolated were phaseollidin (**6**) and phaseollin (**7**) which were identified by comparison with authentic samples (UV, MS, TLC) and 5-deoxykievitone (**11**), an isoflavanone apparently unique to *P. vulgaris* [7]. 6a-Hydroxyphaseollin (**8**) was isolated from extracts of inoculated tissue and was identified by UV, MS, and ^{1}H NMR. The ^{1}H NMR spectrum (in $CDCl_3$ and $(CD_3)_2CO$) differs from the published spectrum of **8** (in CD_3CN) [8] in that the two C-6 protons are non-equivalent. Five minor components were identified as non-prenylated 5-deoxyisoflavonoids as described below.

One of the minor components in the extract (component 1) was identified as daidzein (structure **1a**) by comparison (TLC, MS, UV) with synthetic daidzein. The UV spectrum of component 2 is similar to that of daidzein [9] indicating an isoflavone structure. The MS shows prominent ions at *m/e* 270 (M^+) $[C_{15}H_{10}O_5]^+$, 253 $[C_{15}H_9O_4]^+$, 137 $[C_7H_5O_3]^+$, and 134 $[C_8H_6O_2]^+$. The fragment ions at *m/e* 134 and137 correspond to a retro Diels–Alder (RDA) cleavage and indicate that ring B is dihydroxylated (*m/e* 134) and ring A is monohydroxylated. The abundant fragment ion at *m/e* 253 $[M-OH]^+$ is characteristic of simple 2′-hydroxylated isoflavones [10]. On biogenetic grounds, C-7 and C-4′ are also hydroxylated [11] and the ^{1}H NMR spectrum is consistent with structure **2a** for component 2. The isoflavone C-2 proton appears at δ 8.14 and the six aromatic protons appear as two ABX systems with protons at 6.98 (*d*, C-6′), 6.36 (*d*, C-3′), and 6.27 (*dd*, C-5′) assigned to ring B (compare the corresponding signals for 2′-hydroxygenistein (**14**) at 6.97, 6.36, and 6.25 [12]).

*Part 19 in the series "Studies on Phytoalexins". For Part 18 see Woodward, M. D. (1979) *Phytochemistry* **18**, 2007 .

Table 1. Chromatographic properties and quantitation of *P. vulgaris* isoflavonoids

Compound		TLC systems* ($R_f \times 100$) I	II	III	Chromatography Fraction†	Amount isolated (μmol)‡
5-Deoxyisoflavonoids						
Daidzein	**1a**	41	40	40	C4	1.1
7,2',4'-Trihydroxyisoflavone	**2a**	31	25	33	C5, D1	4.8
7,2',4'-Trihydroxyisoflavanone	**3a**	23	17	38	C4	3.1
5-Deoxykievitone	**11**	28	23	40	B1	3.9
3,9-Dihydroxypterocarpan	**5a**	67	73	48	B1	8.8
Phaseollidin	**6**	76	84	47	P2, A2, B1	73
Phaseollin	**7**	87	89	59	P1, A1	450
6a-Hydroxyphaseollin	**8**	70	78	64	P1	ND
7,2',4'-Trihydroxyisoflavan	**9a**	43	36	37	B2, C3	5.8
Phaseollinisoflavan	**10**	76	84	44	—	—
Coumestrol	**12**	54	50	9	E	ND
5-Hydroxyisoflavonoids						
Genistein	**13**	59	57	26	C6	1.6
2'-Hydroxygenistein	**14**	36	26	25	C6, D2, D3, D4, E	130
Dalbergioidin	**15**	33	20	29	C5, C6, D1, D2	37
Kievitone	**16**	41	29	30	B1, B2, B3	150
1",2"-Dehydrocyclokievitone	**17**	59	53	44	P3	0.43
Licoisoflavone A	**18**	61	58	24	C6, C7, D4	77
2,3-Dehydrokievitone	**19**	54	45	29	B3, C5	6.4

*I: petrol (55–65°)–Et_2O–HOAc, 25:75:1 (0.1 mm Si gel, Eastman 13179, iodine vapour detection); II: petrol (55–65°)–EtOAc–MeOH, 10:10:1 (Si gel); III: MeOH–H_2O, 17:3 (0.05 mm polyamide, Cheng Chin Trading Co., Fast Blue Salt B detection [52]).

†P: petrol fraction; A–E: EtOAc fraction separated on a polyamide column in 85% EtOH; numbers following letters represent column fractions obtained from next purification step.

‡Quantities reported are the amounts isolated from 9 l. of inoculation droplets and are based on literature extinction coefficients (log ε in parentheses): **1a** (4.40) [53], **2a** (4.36) (this study), **6** (3.78) [52], **7** (3.97) [4], **13** (4.55) [53], **15** (4.31) [16], **16** (4.22) [3], and **18** (4.47) [54]. Values for other compounds are based on extinction coefficients for related compounds: **3a** and **11** based on **3b** (4.23) [26], **5a** based on homopterocarpin (3.89) [55], **8** based on **7** (3.97) [4], **9a** on **9b** 3.76 [23], **17** based on cajanone (4.30) [56], and **14** and **19** based on luteone (4.45) [57]. ND = Not determined. — = Not detectable.

The UV spectrum of component 3 shows a 55 nm bathochromic shift with both NaOH and NaOAc which is consistent with a 5-deoxyisoflavanone structure [13, 14]. The MS is simple showing mainly a medium intensity molecular ion at *m/e* 272 $[C_{15}H_{12}O_5]^+$ and major fragment ions at *m/e* 137 $[C_7H_5O_3]^+$ and 136 $[C_8H_8O_2]^+$ which result from a RDA fragmentation and indicate that ring A is monohydroxylated (*m/e* 137) and ring B is dihydroxylated. The ^1H NMR spectrum confirms the isoflavanone nature of component 3 with signals for the three heterocyclic ring protons at δ 4.59 (*t*), 4.44 (*dd*), and 4.09 (*dd*). The pattern observed for protons at C-2 and C-3 in isoflavanones appears to vary with solvent [7] and with substitution. The spectra of the isoflavanones obtained from French bean all show the same pattern (in $(CD_3)_2CO$) as above with complete resolution of the two C-2 protons. Non-equivalence of the C-2 protons is indicative of C-2' substitution [15] although the C-2 protons of kievitone and dalbergioidin were not resolved at 100 MHz (in $(CD_3)_2CO$) [3, 16]. The aromatic protons in the spectrum of component 3 appear as two ABX systems with the protons at 7.74 (C-5), 6.56 (C-6), and 6.39 (C-8) assigned to ring A based on a comparison with the corresponding protons in the spectrum of 7,3'-dihydroxy-4'-methoxyisoflavanone [14]. The chemical shifts of the ring B protons of component 3 are nearly identical to those of dalbergioidin [16] indicating hydroxylation at C-2' and C-4' and that component 3 is 7,2',4'-trihydroxyisoflavanone.

The UV spectrum of component 4 is nearly superimposable on that of phaseollidin (**6**). The pterocarpan nature of the compound is indicated by the ^1H NMR spectrum which gives signals for six aromatic protons (two ABX systems) and for four protons in the heterocyclic ring which appear in a highly complex pattern characteristic of pterocarpans [17]. The MS shows a molecular ion at *m/e* 256 $[C_{15}H_{12}O_4]^+$ which is displaced to 258 in the presence of D_2O indicating that the only substituents are two hydroxyls. These can be assigned to C-3 and C-9 on biogenetic grounds [11] and the following ^1H NMR data. The protons in one of the ABX systems appear at δ 7.39 (*d*), 6.56 (*dd*), and 6.42 (*d*) and these chemical shifts are almost identical to those for the protons at C-1, C-2, and C-4 in the spectrum of medicarpin (**5b**) [18]. The chemical shifts and coupling constants for the ring D protons are nearly equivalent to those reported for **5b** [18] and homopterocarpin [17] indicating substitution at C-9 and that component 4 is 3,9-dihydroxypterocarpan.

ORD spectra of 3,9-dihydroxypterocarpan and

phaseollidin are nearly identical in the 240–350 nm region with each compound showing a positive Cotton effect near 294 nm. These spectra are similar to the spectrum of (-)-homopterocarpin [19] and therefore these pterocarpans are assigned the (6a*R*, 11a*R*)-configuration (**5a** and **6**). The ORD spectra of phaseollin and 6a-hydroxyphaseollin are also nearly identical and each shows a positive Cotton effect in the 335 nm region. Tuberosin, an isomer of 6a-hydroxyphaseollin, shows a negative Cotton effect in this region [20]. The spectra of both phaseollin and 6a-hydroxyphaseollin show large negative troughs in the 240 nm region as do 3,9-dihydroxypterocarpan and phaseollidin, and from this it is concluded that phaseollin has the (6a*R*, 11a*R*)-configuration **7**. 6a-Hydroxyphaseollin is assigned the (6a*S*, 11a*S*)-configuration **8** (opposite to the other pterocarpans which lack the 6a hydroxyl). No rotation was observed in the ORD spectra of any isoflavanone (dalbergioidin, kievitone, 7,2′,4′-trihydroxyisoflavanone, and 5-deoxykievitone) and this is probably the result of the formation of keto–enol tautomers [21].

The remaining non-prenylated isoflavonoid ($C_{15}H_{14}O_4$) was shown to be an isoflavan by the presence of a CH_2-CH-CH_2 group in the ^1H NMR spectrum which is characteristic of isoflavans [19, 22]. These protons appear at δ 4.21 (*ddd*, C-2a), 3.93 (*t*, C-2b), 3.49 (*m*, C-3), 2.94 (*dd*, C-4a), and 2.76 (*ddd*, C-4b) and these shifts are nearly identical to those of the corresponding protons in the spectrum of phaseollinisoflavan dimethylether [3]. Long range coupling was observed between C-2a and C-4b. The molecular ion in the mass spectrum at *m/e* 258 was displaced to *m/e* 261 after D_2O exchange indicating the presence of 3 hydroxyls and prominent fragment ions at *m/e* 136 $[C_8H_8O_2]^+$ and 123 $[C_7H_7O_2]^+$ are the result of a RDA fragmentation with ring B (*m/e* 136) being dihydroxylated. The aromatic protons appear as two ABX systems with coupling constants similar to those of the isoflavone **2a** and the isoflavanone **3a** indicating that the compound is 7,2′,4′,-trihydroxyisoflavan. The chemical shift for each of the six aromatic protons is nearly equivalent to the shift of the corresponding proton in the spectrum of vestitol (**9b**) [23]. The ORD spectrum of 7,2′,4′-trihydroxyisoflavan has a positive Cotton effect at 293 nm and a large negative trough at 239 nm and is assigned the (3*R*)-configuration (structure **9a**) based on the similarity of the spectrum with that of 2′-hydroxy-7,4′-dimethoxyisoflavan [19].

The structures of the natural products **2a**, **3a**, **5a**, and **9a** have been confirmed by synthesis. 7,2′,4′-Trihydroxyisoflavone (**2a**) was prepared from 2,4,4′-tribenzyloxy-2′-hydroxychalcone by oxidative rearrangement with thallium nitrate [24, 25]. Catalytic hydrogenation of the isoflavone triacetate in EtOAc gave racemic 7,2′,4′-triacetoxyisoflavanone from which racemic **3a** was obtained by treatment with base [26]. Racemic pterocarpan **5a** was prepared from **2a** by $NaBH_4$ reduction [26] and racemic isoflavan **9a** was obtained from **2a** by catalytic hydrogenation in HOAc.

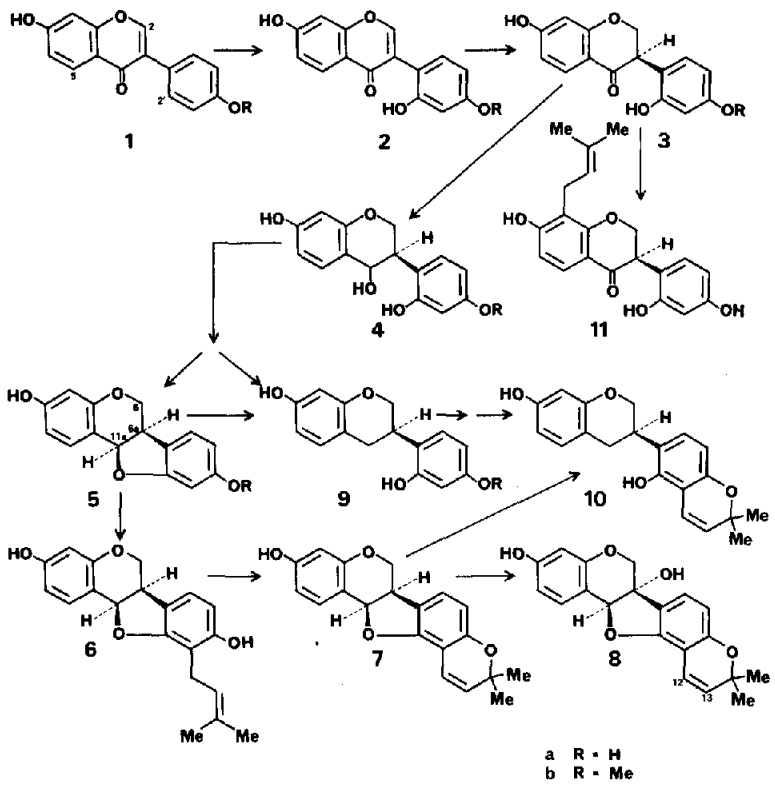
DISCUSSION

Daidzein has been reported from several legume species but not from *P. vulgaris* [27]. The pterocarpan **5a** has been observed previously as a fungal-mediated demethylation product of medicarpin (**5b**) [28] and as a constituent of the heartwood of *Albizzia procera* [29]. The *A. procera* pterocarpan appears to have the (6a*S*, 11a*S*)-configuration. Pterocarpan **8** has been reported as a fungal-mediated hydroxylation product of phaseollin (**7**) [8]. 7,2′,4′-Trihydroxyisoflavan is a post-infectionally formed metabolite of *Anthyllis vulneraria*, *Lotus corniculatus*, *L. uliginosus*, and 5 species of *Tetragonolobus* [30]. However, the configuration of the isoflavan was not reported. Neither the isoflavone **2a** nor the isoflavanone **3a** has been reported as naturally occurring.

With the exception of **9a**, the non-prenylated 5-deoxyisoflavonoids which have now been characterized from *P. vulgaris* probably constitute the isoflavonoid intermediates involved in phaseollidin and phaseollin biosynthesis. This indicates that the sequence of reactions **1a** → **2a** → **3a** → **4a** → **5a** → **6** → **7** (Scheme 1) probably occurs during phaseollin formation. Other than 5-deoxykievitone (**11**) and 6a-hydroxyphaseollin (**8**), no additional 5-deoxyisoflavonoids were identified from the extracts. These observations are consistent with the postulate that non-prenylated isoflavonoids are intermediates in phaseollin biosynthesis. The sequence shown in Scheme 1 for phaseollin formation (isoflavone formation, 2′-hydroxylation, reduction to an isoflavanone and then to an isoflavanol followed by dehydration and cyclization to a pterocarpan) is the same as that proposed for medicarpin biosynthesis (Scheme 1, R = methyl) based on an elegant set of experiments using labelled substrates [26, 31–33]. Isolated 7,2′,4′-trihydroxyisoflavanone and 5-deoxykievitone were optically inactive probably as a result of the formation of keto–enol tautomers [21]; however, they are depicted in Scheme 1 (structures **3a** and **11**) with the same configuration (3*R*) as found for the pterocarpans. The isoflavanol **4b** has been proposed as an intermediate in medicarpin biosynthesis [26]. The isoflavanol **4a** was not isolated in this study but this was not unexpected as such compounds readily undergo cyclization to give pterocarpans [34]. The interconversion of the isoflavan vestitol (**9b**) and the pterocarpan medicarpin (**5b**) has been demonstrated in *Medicago sativa* [35, 36] and this observation led to a postulation that these compounds were formed from a common intermediate. A similar system may exist in *P. vulgaris* as both the isoflavan **9a** and the pterocarpan **5a** were isolated.

The ultimate step in the formation of phaseollidin (**6**) appears to be prenylation of the pterocarpan **5a**. This proposal is based on the absence of prenylated isoflavones and isoflavanones which could serve as precursors. The biosynthesis of other prenylated pterocarpans may be similar, as for example, in the case of *Glycine max* where the principal phytoalexin is the prenylated pterocarpan glyceollin [37]. 3,6a,9-Trihydroxypterocarpan has been isolated from $CuCl_2$-treated *G. max* cotyledons [38] and recently an enzyme has been obtained from *G. max* which prenylates the pterocarpan [39]. It seems very probable that both the non-prenylated pterocarpan and the prenylated product are precursors of glyceollin.

The enzymes responsible for hydroxylation and prenylation of the French bean isoflavonoids appear to have a high level of specificity. Hydroxylation appears



Scheme 1. Proposed pathway for the biosynthesis of phaseollin and related 5-deoxyisoflavonoids in *P. vulgaris* (R = H).

to occur specifically at the 2′-position of the isoflavones genistein and daidzein. Of the ten compounds isolated from French bean showing prenylation , only positions C-8 and C-3′ (C-10 for pterocarpans) are prenylated. The formation of 5-deoxykievitone probably follows the sequence **1a** → **2a** → **3a** → **11** (Scheme 1). Although a specific enzyme may exist for the prenylation of **3a**, it is likely that if an enzyme exists that prenylates 5,7,2′,4′-tetrahydroxyisoflavanone (dalbergioidin) in position C-8 to give kievitone this enzyme could probably prenylate **3a** with lower efficiency resulting in the accumulation of **11**.

Phaseollinisoflavan (**10**) was not isolated from the inoculation fluid in this study. Formation of phaseollinisoflavan has been reported from *P. vulgaris* cell suspension cultures in response to the addition of phaseollin to the medium [40], from filtrates of fungal cultures grown in the presence of phaseollin [41], and from several cultivars of *P. vulgaris* including Red Kidney following inoculation with bean pathogens [42–47]. The absence of **10** in the inoculation fluid from the interaction of *P. vulgaris* with *M. fructicola*, a non-pathogen of beans, could be due to the short incubation period used, or to the fungal species used, or, if **10** was present, it was either in amounts below the level of detection or was lost during the isolation procedures. Formation of **10** in the plant could occur directly from **7** [40] or from **9a** via the intermediate phaseollidinisoflavan. However, this latter compound has not been reported from French beans.

The coumestan, coumestrol, has been reported to occur in *P. vulgaris* after treatment with culture filtrates of *Penicillium expansum* [48] and with bacteria [49]. Chromatographic evidence (co-chromatography on polyamide, Si gel, and cellulose with detection by fluorescence) was obtained in this study for the presence of a coumestrol-like compound in inoculated bean tissue and in the inoculation fluid; however, no attempt was made to purify the material.

EXPERIMENTAL

Low resolution MS was obtained using a direct insertion probe (ionization voltage 70 eV; accelerating voltage 4 kV) and precise mass measurements were obtained using an on-line computer. All ^{1}H NMR spectra were recorded at 270 MHz using TMS as internal standard. ORD spectra were recorded in MeOH at 25° and concentrations used are based on ε values reported in Table 1.

Isolation of isoflavonoids from inoculation droplets. Inoculation of *P. vulgaris* L. cv. Red Kidney pod cavities (*ca* 40 000) with a conidial suspension (*ca* 0.4×10^6 spores/ml) of *Monilinia fructicola* (Wint.) Honey [4] gave 9.5.1 of inoculation fluid after a 20 hr incubation period and the processing and sequential extraction of this material with petrol and EtOAc has been described [7]. Five fractions were obtained after chromatography of the EtOAc-extractable material on a column of polyamide eluted with 85% EtOH and the distribution of the compounds is shown in Table 1. Fraction A was chromatographed on a column of Si gel eluted with $CHCl_3$ to obtain phaseollin and phaseollidin. Fraction B was rechromatographed on polyamide in 85%

EtOH. Fraction B-1 contained all of **5a** which was purified by chromatography on polyamide (70% MeOH) then Si gel $CHCl_3$ using a 0–3% MeOH gradient. Fraction C-4 con- (95% EtOH). Fraction C was chromatographed on a column of polyamide eluted with 85% MeOH and each of the 7 fractions was then chromatographed on Si gel eluted with $CHCl_3$ using a 0–3% MeOH gradient. Fraction C-4, contained **1a** and **3a** which were separated on the Si gel column (**1a** at *ca* 2% MeOH, **3a** at 3% MeOH) and each compound was further purified by chromatography on an LH-20 column in 95% EtOH. Fraction B-2 and fraction C-3 contained **9a** and were combined and chromatographed on an LH-20 column in 95% EtOH. Fraction D was chromatographed on polyamide in 85% MeOH and all 4 fractions obtained were chromatographed on Si gel columns in $CHCl_3$ and eluted with a 0–3% MeOH gradient in $CHCl_3$. Compound **2a** was obtained from fractions C-5 and D-1 at *ca* 2% MeOH. The appropriate fractions were pooled and chromatographed on an LH-20 column in 95% EtOH to give **2a**. All other compounds listed in Table 1 were isolated using columns of polyamide (70–85% MeOH), Si gel ($CHCl_3$–MeOH gradient), and LH-20 (95% EtOH) and then pooling the appropriate fractions. Compounds **6**, **7**, and **17** were obtained from the petrol fraction by chromatography on columns of polyamide (70% MeOH) and LH-20 (95% EtOH) [7].

7,2',4'-Trihydroxyisoflavone (**2a**). UV λ_{max}^{MeOH} nm; 241 (sh), 248, 258 (sh), 290. ^{1}H NMR ($(CD_3)_2SO$): δ 8.14 (1H, *s*, C-2); 7.93 (1H, *d*, *J* = 8.8 Hz, C-5); 6.98 (1H, *d*, J = 8.3 Hz, C-6'); 6.93 (1H, *dd*, *J* = 8.8, 2.2 Hz, C-6); 6.86 (1H, *d*, *J* = 2.2 Hz, C-8); 6.36 (1H, *d*, *J* = 2.4 Hz, C-3'); 6.27 (1H, *dd*, *J* = 8.3, 2.4 Hz, C-5'). MS, M^+ 270.0527, $C_{15}H_{10}O_5$ requires 270.0528, *m/e* (rel. int.): 270 (100), 269 (18), 253 (41), 137 (68), 135 (11), 134 (40).

Synthesis of **2a**. 2,4-Dibenzyloxybenzaldehyde (6.36 g) and 2-hydroxy-4-benzyloxyacetophenone (4.84 g) were dissolved in EtOH (60 ml), 10 ml 50% NaOH was added, and the mixture was heated on a steam bath for 20 min and then filtered [24]. The product was washed with 0.1 N HCl and then H_2O until neutral. Crystallization from HOAc gave 2,4,4'-tribenzyloxy-2'-hydroxychalcone (5.60 g, 52%, mp 127–130°, lit. 141–142° [25]). MS *m/e* (rel. int.): 542 (M^+, 75), 452 (31), 451 (85), 435 (62), 434 (100), 253 (46), 227 (79), 181 (32). The chalcone (2.71 g) was added to MeOH (1 l.), stirred and heated to 50°, $Tl(NO_3)_3 \cdot 3H_2O$ (2.44 g) was added, and stirring continued for 2.5 hr. Then 30 ml 3N HCl was added, the mixture was heated under reflux on a steam bath for 7 hr, filtered hot, and concd under red. pres. [24]. H_2O was added, the mixture was stirred at 4° for 3 hr, and then filtered. The crude product from two such syntheses was pooled and crystallized from $CHCl_3$–MeOH to give 7,2',4'-tribenzyloxyisoflavone (4.04 g, 75%, mp 156–158°, lit. 154–157° [25]). MS *m/e* (rel. int.): 540 (M^+, 97), 450 (50), 449 (100), 359 (13), 181 (22), 120 (64), 119 (33), 118 (69), 117 (36). Catalytic hydrogenation of the above isoflavone (3.78 g) with Pd–charcoal (5%, 0.5 g) in 150 ml Me_2CO at room temp. and 3 atm for 12 hr gave **2a** which was crystallized from aq. MeOH (1.19 g, 63%). The colourless isoflavone was obtained by chromatography on Si gel using a 0–4% MeOH gradient in $CHCl_3$ and crystallization from $CHCl_3$–MeOH gave the desired product (mp 275° dec., lit. 272° dec. [50], lit. 284° dec. [51]). (Found: C, 66.45; H, 3.75. Calc. for $C_{15}H_{10}O_5$: C, 66.65; H, 3.73%.) MS and ^{1}H NMR as for isolated material. UV λ_{max}^{MeOH} nm (log ε): 241 (4.33), 249 (4.36), 259 (sh) (4.27), 289 (4.15). The synthetic compound was indistinguishable from the natural product by TLC (8 solvent systems).

7,2',4'-Trihydroxyisoflavanone (±) (**3a**). UV λ_{max}^{MeOH} (nm): 278, 308 (sh); NaOH 246, 333; $AlCl_3$ 277, 309; $AlCl_3$–HCl 277, 305 (sh); NaOAc 254, 289, 333; NaOAc–H_3BO_3 279, 313. ^{1}H NMR ($(CD_3)_2CO$): δ 7.74 (1H, *d*, *J* = 8.8 Hz, C-5); 6.81 (1H, *d*, *J* = 8.3 Hz, C-6'); 6.56 (1H, *dd*, *J* = 8.8, 2.2 Hz, C-6); 6.45 (1H, *d*, *J* = 2.5 Hz, C-3'); 6.39 (1H, *d*, *J* = 2.2 Hz, C-8); 6.24 (1H, *dd*, *J* = 8.3, 2.5 Hz, C-5'); 4.59 (1H, *t*, *J* = 10.7 Hz, (C-2a); 4.44 (1H, *dd*, *J* = 10.8, 5.4 Hz, C-2b); 4.09 (1H, *dd*, *J* = 10.6, 5.4 Hz, C-3). MS, M^+ 272.0690, $C_{15}H_{12}O_5$ requires 272.0685; *m/e* (rel. int.): 272 (31), 137 (100), 136 (36), 135 (8).

Synthesis of (±) **3a**. Isoflavone **2a** (1.08 g) in C_5H_5N (3 ml) was treated with Ac_2O (3 ml) at 70° for 90 min, poured into H_2O, and the product filtered to give 7,2',4'-triacetoxyisoflavone (1.41 g, 89%). A portion was chromatographed on Si gel in $CHCl_3$ and recrystallized from MeOH (mp 149–151°, lit. 148–150° [50]). (Found: C, 63.33; H, 4.25. Calc. for $C_{21}H_{16}O_8$: C, 63.62; H, 4.07%). MS *m/e* (rel. int.): 396 (M^+, 24), 354 (53), 312 (100), 270 (96), 269 (15), 253 (16), 137 (26), 134 (17). The acetylated isoflavone (79 mg) was hydrogenated using Pd–charcoal (5%, 100 mg) in EtOAc (20 ml) at room temp. for 16 hr [26]. After filtration, the crude product from four such reactions was chromatographed on Si gel in $CHCl_3$ to give 7,2',4'-triacetoxyisoflavanone which crystallized from EtOH (125 mg, 39%, mp 118–119°). (Found: C, 63.52; H, 4.35. $C_{21}H_{18}O_8$ requires: C, 63.30; H, 4.56%). MS *m/e* (rel. int.): 398 (M^+, 1.4), 356 (24), 338 (26), 314 (17), 179 (68), 178 (24), 137 (100), 136 (71), 135 (12). The above isoflavanone (27 mg) was treated with 0.5 g KOH in 10 ml EtOH for 3 hr [26]. The reaction mixture was poured into H_2O (140 ml), neutralized with 2 N HCl, and extracted with EtOAc (150 ml, 2×). The product **3a** was purified on Si gel eluted with a 0–4% MeOH gradient in $CHCl_3$ (*ca* 18 mg, 97%). UV λ_{max}^{MeOH} (nm): 277, 309; MS and ^{1}H NMR as for natural product. The isolated and synthetic materials were indistinguishable by TLC (10 solvent systems).

(6*a*R, 11*a*R)-3,9-*Dihydroxypterocarpan* (**5a**). UV λ_{max}^{MeOH} (nm): 286, 282 (sh). ^{1}H NMR ($CDCl_3$): δ 7.39 (1H, *d*, *J* = 8.4 Hz, C-1); 7.08 (1H, *d*, *J* = 8.0 Hz, C-7); 6.56 (1H, *dd*, *J* = 8.4, 2.4 Hz, C-2); 6.42 (1H, *d*, *J* = 2.4 Hz, C-4); 6.40 (1H, *dd*, *J* = 8.0, 2.0 Hz, C-8); 6.37 (1H, *d*, *J* = 2.0 Hz, C-10); 5.49 (1H, *d*, *J* ~ 6.2 Hz, C-11a); 4.23 (1H, *m*, C-6eq); 3.62 (1H, *t*, *J* ~ 10.6 Hz, C-6ax); 3.49 (1H, *m*, C-6a). MS, M^+ 256.0737, $C_{15}H_{12}O_4$ requires 256.0736; *m/e* (rel. int.): 256 (100), 255 (49), 239 (7), 147 (23), 134 (21), 128 (11), 123 (7). ORD (*c* 0.10 mM, *l* 2 cm): $[\phi]_{312}$ 0°, $[\phi]_{295}$ + 11 000°, $[\phi]_{288}$ 0°, $[\phi]_{278}$ − 21 000°, $[\phi]_{267}$ − 19 000°, $[\phi]_{242}$ − 49 000°. Treatment of **5a** with CH_2N_2 gave a dimethylether which was identical to homopterocarpin by MS. MS *m/e* (rel. int.): 284 (100), 283 (38), 269 (29), 161 (19), 142 (13).

Synthesis of (±) **5a**. Isoflavone **2a** (108 mg) in dry THF was stirred and $NaBH_4$ (0.15 g) suspended in EtOH was added slowly. The mixture was stirred for 2 days, Me_2CO was added, and the solvents were evapd [26]. The residue was treated with 3 N HCl (20 ml) and extracted 3× with EtOAc (20 ml). The product was obtained in low yield [50] and was separated from the unreacted starting material by chromatography on polyamide (85% MeOH) and on LH-20 in 95% EtOH (*ca* 15 mg, 14%). UV, ^{1}H NMR (in $CDCl_3$), and MS as for natural product. ^{1}H NMR ($(CD_3)_2CO$): δ 7.32 (1H, *d*, *J* = 8.3 Hz, C-1); 7.13 (1H, *d*, *J* = 8.0 Hz, C-7); 6.56 (1H, *dd*, *J* = 8.3, 2.4 Hz, C-2); 6.37 (1H, *dd*, *J* = 8.1, 2.2 Hz, C-8); 6.36 (1H, *d*, *J* = 2.4 Hz, C-4); 6.29 (1H, *d*, *J* = 2.2 Hz, C-10); 5.46 (1H, *d*, *J* = 5.9 Hz, C-11a), 4.24 (1H, *m*, C-6eq); 3.56

(2H, *m*, C-6a, C-6ax). The synthetic material was indistinguishable from the natural product from French beans and from the fungal demethylation product of medicarpin [28] by TLC in 6 solvent systems.

(6*a*R,11*a*R)-*Phaseollidin* (**6**). UV and MS as literature [2]. ORD (*c* 0.10 mM, *l* 2 cm): $[\phi]_{312}$ 0°, $[\phi]_{292}$ +14 000°, $[\phi]_{287}$ 0°, $[\phi]_{276}$ −19 000°, $[\phi]_{268}$ −18 000°, $[\phi]_{244}$ −33 000°.

(6*a*R,11*a*R)-*Phaseollin* (**7**). UV and MS as literature [2]. ORD (*c* 0.06 mM, *l* 2 cm): $[\phi]_{336}$ +4400°, $[\phi]_{322}$ 0°, $[\phi]_{303}$ −5200°, $[\phi]_{291}$ −2000°, $[\phi]_{272}$ −18 000°, $[\phi]_{240}$ −41 000°.

(3R)-7,2′,4′-*Trihydroxyisoflavan* (**9a**). UV λ_{max}^{MeOH} nm: 283. ^{1}H NMR ($(CD_3)_2CO$): δ 6.91 (1H, *d*, A), 6.47 (1H, *d*, X), 6.30 (1H, *dd*, B), J_{AB} = 8.4, J_{BX} = 2.4 Hz; 6.87 (1H, *d*, A′), 6.35 (1H, *dd*, B′), 6.27 (1H, *d*, X′), $J_{A'B'}$ = 8.3 Hz, $J_{B'X'}$ = 2.4 Hz; 4.21 (1H, *ddd*, *J* = 10.2, 3.8, 2.2 Hz, C-2a); 3.93 (1H, *t*, *J* = 10.2 Hz, C-2b); 3.49 (1H, *m*, C-3); 2.94 (1H, *dd*, *J* = 15.4, 11.0 Hz, C-4a); 2.76 (1H, *ddd*, *J* = 15.4, 5.2, 2.2 Hz, C-4b). MS, M^+ 258.0880, $C_{15}H_{14}O_4$ requires 258.0892; *m/e* (rel. int.): 258 (59), 147 (9), 137 (15), 136 (100), 135 (39), 134 (23), 124 (21), 123 (73), 107 (14). ORD (*c* 0.15 mM, *l* 2 cm): $[\phi]_{293}$ +1800°, $[\phi]_{289}$ 0°, $[\phi]_{278}$ −2700°, $[\phi]_{263}$ −1200°, $[\phi]_{239}$ −5600°.

Synthesis of (±) **9a**. Isoflavone **2a** (108 mg) was hydrogenated in HOAc (40 ml) using Pd-charcoal (5%, 200 mg) at room temp. for 65 hr at 3 atm. The product was purified by chromatography on Si gel using a 0–4% MeOH gradient in $CHCl_3$ and then on LH-20 in 95% EtOH to give 7,2′,4′-trihydroxyisoflavan (*ca* 13 mg, 13%). UV, ^{1}H NMR, and MS as for isolated material and the two compounds were identical by TLC (8 solvent systems).

Isolation of (6*a*S,11*a*S)-6*a*-*hydroxyphaseollin* (**8**) *from inoculated tissue. P. vulgaris* endocarp tissue (3.75 kg) under the inoculation droplets was handled as described previously [6]. The methanolic soln obtained was chromatographed on a column of polyamide in 85% MeOH and **8** was eluted with **6** and **7**. Subsequent separation on a column of polyamide eluted with 60% MeOH gave **8** as the first peak to elute followed by **7** and then **6**. Further purification of the fraction containing **8** was performed by chromatography on an LH-20 column in 95% EtOH. UV λ_{max}^{MeOH} (nm): 280, 285 (sh), 311 (sh). ^{1}H NMR ($(CD_3)_2CO$): δ 7.34 (1H, *d*, *J* = 8.5 Hz, C-1); 7.14 (1H, *d*, *J* = 8.2 Hz, C-7); 6.56 (1H, *dd*, *J* = 8.5 2.4 Hz, C-2); 6.42 (1H, *d*, *J* = 9.8 Hz, C-12); 6.34 (1H, *d*, *J* = 8.2 Hz, C-8); 6.33 (1H, *d*, *J* = 2.2 Hz, C-4); 5.67 (1H, *d*, *J* = 10.0 Hz, C-13); 5.34 (1H, *s*, C-11a); 4.13 (1H, *d*, *J* = 11.4 Hz, C-6); 4.06 (1H, *d*, *J* = 11.4 Hz, C-6); 1.39 (3H, *s*, methyl); 1.35 (3H, *s*, methyl). MS, M^+ 338.1155, $C_{20}H_{18}O_5$ requires 338.1154; *m/e* (rel. int.): 338 (41), 323 (100), 320 (18), 305 (29), 295 (25), 185 (11), 161 (33). ORD (*c* 0.06 mM, *l*, 2 cm): $[\phi]_{343}$ 0°, $[\phi]_{332}$ +2200°, $[\phi]_{322}$ 0°, $[\phi]_{304}$ −4300°, $[\phi]_{291}$ −2400, $[\phi]_{270}$ −19 000°, $[\phi]_{243}$ −43 000°.

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