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PHASEOLUTEONE AND OTHER 5-HYDROXYISOFLAVONOIDS FROM *PHASEOLUS VULGARIS**

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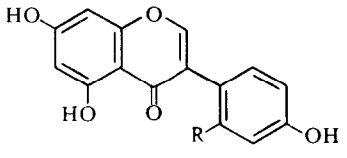
Key Word Index—*Phaseolus vulgaris*; Leguminosae; French bean; isoflavone; isoflavanone; phytoalexin.

Several antifungal isoflavanoids have been found in French bean, *Phaseolus vulgaris* L., after fungal infection [1]. These compounds are believed to play a primary role in stopping fungal growth *in vivo;* however, little is known about their formation in the bean plant. The elucidation of the biosynthetic pathways of these post-infectionally formed metabolites is required for an understanding of the defence mechanisms of the plant. Kievitone **5** is unique among the French bean antifungal isoflavanoids in the dihydroxylation of ring A [2, 3]. This paper describes the isolation and identification of a new isoflavone, phaseoluteone **3**, which is also dihydroxylated in ring A, and the isolation of three additional isoflavonoids which may be precursors of kievitone and/or phaseoluteone.

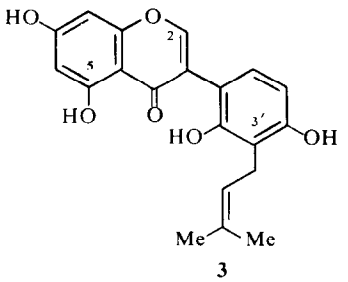
In an examination of some of the unidentified phenolic compounds detected in the interaction between French bean pod tissue and the fungus *Monilinia fructicola* (Wint.) Honey, three 5,7-dihydroxyisoflavones and two 5,7-dihydroxyisoflavanones were identified. The isoflavone genistein **1** and the isoflavanone dalbergioidin **4** [4] were identified by UV, MS, and TLC comparisons with authentic samples. Kievitone was identified by comparison of the UV, PMR, and MS data with those in the literature [2, 3]. The fourth compound was tentatively identified as 2′-hydroxygenistein **2** based on UV, PMR, and MS data [5–7]. The identification was confirmed by preparation of a phenolic trimethyl ether which was identical to an authentic sample of 5-hydroxy-7,2′,4′-trimethoxyisoflavone [8] by UV, MS, and TLC.

The fifth compound is a new isoflavone **3** for which the trivial name phaseoluteone is suggested. From high resolution mass spectrometry, phaseoluteone has a molecular formula of $C_{20}H_{18}O_6$. Acetylation produced a tetraacetate (M^+ 522) indicating the presence of four hydroxyl groups. Bathochromic shifts in the UV spectrum of **3** induced by NaOH, $AlCl_3$, and NaOAc are consistent with its formulation as an isoflavone hydroxylated at C-5 and C-7 [9]. The PMR signal at δ 13.11 (1H, *s*, exchangeable in D_2O) is characteristic of the 5 OH in isoflavones [10]. The isoflavone C-2 appears as a characteristic downfield singlet [11, 12] and was observed at δ 8.12. Signals for four aromatic protons were observed. The two higher field protons (δ 6.33, 6.46) are a *meta* coupled pair (J = 2.0 Hz) and are assigned to positions 6 and 8 of ring A (compare the corresponding protons for genistein δ 6.27, 6.39; 2′-hydroxygenistein δ 6.29, 6.43 and 5,7,2′-trihydroxy-4′-methoxyisoflavone [8] δ 6.35, 6.48). The UV and PMR data are consistent with ring A substitutions at C-5 and C-7 only. The two lower field protons (δ 6.90, 6.54) appear as an *ortho* pair (J = 8.5 Hz). Since nearly all naturally occurring isoflavones are oxygenated at C-4′ [12, 13], these protons are assignable to C-6′ and C-5′ (compare the respective protons of auriculatin δ 6.96, 6.50 [14] and koparin δ 6.70, 6.48 [15]). Signals at δ 1.64 (3H, *s*), 1.78 (3H, *s*), 3.44 (2H, *d*, J = 7.0 Hz) and 5.33 (1H, *br t*, J = 6.8 Hz) indicate the presence of a 3,3-dimethylallyl group. The three substituents on ring B are thus seen to be two hydroxyls and one 3,3-dimethylallyl group. On biogenetic grounds, C-4′ is hydroxylated which leaves two possible arrangements of substituents on ring B. Two lines of evidence confirm the correct structure as **3**. First, the mass spectrum of phaseoluteone shows an $[M - 17]^+$ fragment at *m/e* 337 (6%) which is characteristic of 2′-hydroxylated isoflavones [6, 8, 16]. Second, treatment of phaseoluteone with acid produced two 2,2-dimethylchroman isomers which were differentiated on the basis of their $[M - 17]^+$ fragments.

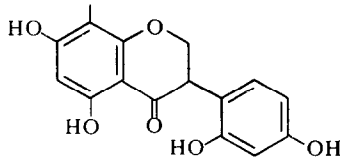
Fragment ions in the mass spectrum of phaseoluteone are consistent with structure **3**. The ion at *m/e* 153 would be expected from a retro-Diels-Alder fragmentation with hydrogen transfer and the charge retained on the dihydroxylated ring A [17]. The corresponding fragment ion for ring B at *m/e* 202 was not observed.



1 R = H
2 R = OH



3



4 = R = H
5 = R = $CH_2CH{=}CMe_2$

* Part 16 in the series "Studies on Phytoalexins". For Part 15 see (1974) *Aust. J. Chem.* **27**, 1607.

Abundant fragments at *m/e* 311 (70%) and 299 (100%) indicate the facile loss of C_3H_7 and C_4H_7 from the 3,3-dimethylallyl group (supported by the presence of second field free region metastable peaks—m* = 273.5, 252.0). The fragment ion at *m/e* 299 gives rise to a fragment ion at *m/e* 147 (*m** = 72.3). The latter fragment would correspond to the retro-Diels-Alder fragmentation with charge retention on the dihydroxylated ring B.

Although simple naturally occurring 2′-hydroxyisoflavones have characteristically large $[M - 17]^+$ fragments (*ca* 20–30%) [6, 8], the corresponding fragment in phaseoluteone is small (6%). This may be due to the presence of the 3,3-dimethylallyl group in the molecule, since luteone, 5,7,2′,4′-tetrahydroxy-6-(3,3-dimethylallyl) isoflavone, also has a small $[M - 17]^+$ fragment (6%).

Dalbergioidin has been reported from only *Ougeinia dalbergioides* Benth. [7] and *Lablab niger* Medik. [18]. 2′-Hydroxygenistein was first reported from *P. vulgaris* pod inoculation droplets [5] and has recently been reported from *Cajanus cajan* (L.) Millsp. [6], *Flemingia macrophylla* (Willd.) Merr. [19], *Glycine wightii* (Wight & Arn.) Verdc. [20], and *L. niger* [18]. The isoflavone phaseoluteone has not been previously reported from any plant although its isomer luteone has been reported from *Lupinus luteus* L. [21] and *Lupinus* spp. [22].

The co-occurrence of compounds **1**–**5** in bean tissue, together with the relative amounts of each compound obtained, may have particular significance for biosynthetic studies. Although 4,2′,4′,6′-tetrahydroxychalcone was not isolated, the primary isoflavonoid expected from this chalcone would be the 5-hydroxyisoflavone genistein **1** [12]. Formation of phaseoluteone **3** probably follows the sequence **1** → **2** → **3**. Similarly, the most direct route to kievitone would be **1** → **2** → **4** → **5**. The same route for kievitone formation has been recently proposed based on similar findings in *L. niger* [18]. Although other pathways may be postulated for the formation of phaseoluteone and kievitone, the failure to detect any other 5,7-dihydroxylated isoflavone or isoflavanone tends to support the proposed pathways.

EXPERIMENTAL

TLC solvent systems used were: I MeOH–H_2O (17:3), II MeOH–H_2O (3:1), III Me_2CO–H_2O (99:1), IV HOAc–H_2O (1:3), and V $CHCl_3$–EtOH (97:3), and I–III were used with polyamide, IV with cellulose, and V with Si gel. Chromatograms were sprayed with Fast Blue Salt B [23]. MS (direct insertion probe; ionisation voltage 70 ev; accelerating voltage 8 KV).

Isolation and purification. P. vulgaris cv Red Kidney pod cavities were prepared and inoculated with a conidial suspension of *Monilinia fructicola* (Wint.) Honey [24]. After 20 hr incubation, the inoculation droplets were collected (and frozen, but not used in this study) and the endocarp tissue (500 g) underlying the droplets was removed and placed in chilled MeOH (1.5 l.). The tissue was stored at −20° for several days, the MeOH was filtered, and additional MeOH (1.5 l.) was added (2×). The 3 methanolic solns were combined, the MeOH was removed *in vacuo* below 40°, and the aq. concentrate (pH 5.8) made up to 200 ml by addition of H_2O. The concentrate was partitioned (6×) against equal vols. of petrol then against equal vols. of EtOAc (3×), and then discarded. Each organic fraction was taken to dryness *in vacuo*. The petrol fraction was dissolved in 3 ml 90% MeOH and partitioned 3× against 3 ml petrol and the petrol was discarded. The methanolic soln was chromatographed on a column of perlon-type polyamide eluted with 85% MeOH. Major phenolic peaks eluted at: 1.5, 2.0, 3.3, and 6.0 column vols and contained mostly phaseollin [24], phaseollidin [25], kievitone, and phaseoluteone, respectively. Fractions were pooled accordingly and rechromatographed on a polyamide column eluted with 85% MeOH. Dalbergioidin was isolated from the tail of the kievitone-containing fraction and from the front of the phaseoluteone-containing fraction. Each fraction was further purified by chromatography on Sephadex® LH-20 in redistilled 95% EtOH. The EtOAc fraction was chromatographed successively on polyamide and LH-20 in the same manner. Each compound was judged to be pure by TLC. Based on published extinction coefficients [2, 4, 26], amounts (μmol) obtained were: **1** 0.31, **2** 3.53, **3** 9.19, **4** 0.38, and **5** 20.7. Compounds **2** and **3** were estimated based on the extinction coefficient of luteone [21]. None of the 5 isoflavonoids were detected in uninoculated tissue by TLC (detection limit < 100 ng/g fr. wt).

Compounds **1**, **2**, **4** *and* **5**. UV maxima in MeOH and all phenolic shift reagents [9], MS, and PMR (**2** and **5** only) were identical to literature values [2–7]. Compounds **1** and **4** were indistinguishable from authentic samples in TLC systems I–IV. Methylation of **2** gave a trimethyl ether which was identical to 5-hydroxy-7,2′,4′-trimethoxyisoflavone [8], by direct comparison. UV λ_{max}^{EtOH} (nm) 260, 281 (sh), 321 (sh); MS: *m/e* (rel. int.) 328 (M^+, 100), 313 (8), 310 (9), 297 (37), 282 (7), 167 (40), 164 (29), 162 (20), 161 (23); TLC: R_f 0.68 and 0.49 in systems I and II, respectively.

5,7,2′,4′-Tetrahydroxy-3′-(3,3-dimethylallyl)isoflavone **3** *(phaseoluteone).* UV λ_{max}^{MeOH} (nm) 210, 263; NaOH 217, 278, 331; $AlCl_3$ 270, 307, 376; $AlCl_3$/HCl 271, 305 (sh), 376; NaOAc 272, 316 (sh); NaOAc/H_3BO_3 262; PMR (100 MHz, $(CD_3)_2CO$) δ 13.11 (1H, *s*, 5-OH); 8.12 (1H, *s*, C-2); 6.90 (1H, *d*, *J* = 8.6 Hz, C-6′); 6.54 (1H, *d*, *J* = 8.5 Hz, C-5′); 6.46 (1H, *d*, *J* = 2.0 Hz, C-8); 6.33 (1H, *d*, *J* = 2.0 Hz, C-6); 5.33 (1H, *br t*, *J* = 6.8 Hz, olefinic); 3.44 (2H, *d*, *J* = 7.0 Hz, methylene); 1.78 (3H, *s*, methyl); 1.64 (3H, *s*, methyl); IR ($CHCl_3$) C=O 1658 cm^{-1}; MS: *m/e* (rel. int.) 354 (M^+, 100), 353 (6), 339 (10), 337 (6), 312 (14), 311 (70), 300 (20), 299 (100), 298 (44), 169 (5), 162 (11), 153 (29), 147 (11). TLC: R_f 0.25, 0.12, 0.62, 0.63, and 0.53 in systems I–V, respectively. The tetraacetate had UV λ_{max}^{EtOH} (nm) 212, 243, 294; MS: *m/e* 522 (M^+, 4), 481 (22), 480 (61), 439 (22), 438 (83), 437 (78), 397 (24), 396 (100), 395 (83), 354 (33), 353 (72), 341 (57). Treatment of phaseoluteone with HOAc and conc H_2SO_4 [21] gave 2 isomers of phaseoluteone—A and B which were separated on a polyamide column eluted with 70% MeOH. Isomer A appears to have a free 4′-OH based on MS: *m/e* 354 (M^+, 75), 339 (6), 337 (0), 312 (5), 311 (24), 300 (14), 299 (100), 298 (36); TLC: R_f 0.43 in system I. Isomer B appears to have a free 2′-OH based on MS: *m/e* 354 (M^+, 95), 339 (11), 337 (7), 312 (11), 311 (36), 300 (18), 299 (100), 298 (45); TLC: R_f 0.36 in system I.

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A NEW ISOFLAVONE GLUCOSIDE FROM *CAJANUS CAJAN*

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Previous investigations [1, 2] of the genus *Cajanus* have established the structures of some new prenylated flavonoids. We now report the isolation and identification of a new isoflavone glucoside, together with 5,7,4′-trihydroxyisoflavone (genistein), and the triterpenoids sitosterol and its glucoside, lupeol, and α- and β-amyrin.

The Me_2CO-insoluble portion of the alcoholic extract of the root bark on column chromatography over Si gel gave two fractions. PC (Whatman 3 mm) of the first fraction using 10% aqueous HOAc yielded the new glucoside as colourless needles (from dilute alcohol) mp 200–203° (d); PC, R_f: 0.89 (BAW, 4:1:5); 0.42 (10% aq. HOAc); TLC, R_f: 0.51 (Si gel, EtOAc–MeOH–H_2O, 100:16.5:13.5). It gave an olive green colour with alcoholic $FeCl_3$. IR(KBr) showed strong absorptions at 1666 cm^{-1} (chelated >C=O) and 3573 cm^{-1}. MS exhibited M^+ 432.1056 ($C_{21}H_{20}O_{10}$) and an aglycone peak at *m/e* 270 (100%) formed by the elimination of a six carbon sugar. These observations coupled with the UV spectrum (λ_{max}^{MeOH} 260, 320; +$AlCl_3$, 240, 270, 365; +$AlCl_3$–HCl 240, 270–75, 365; +NaOAc, 260 nm) indicated it to be an isoflavone glycoside. Acid hydrolysis gave an aglycone which crystallized from MeOH as long straw yellow coloured needles mp 222–24° sintering at 190°; TLC, R_f: 0.52 (Si gel, C_6H_5Me–HCO_2Et–HCO_2H, 5:4:1); 0.78 (Si gel, EtOAc–C_6H_6, 3:7); M^+ 270.0524, $C_{15}H_{10}O_5$; and a sugar unit, identified as glucose by PC. In the MS of the aglucone, the retro–Diels-Alder cleavage produces two ketene fragments at *m/e* 153 (88) and 152 (80), and one acetylene fragment at *m/e* 118 (78) suggesting it to be an isoflavone with two hydroxyls in ring A and one hydroxyl in ring B [3]. The fragment at *m/e* 253 (23), indicated the presence of the hydroxyl in ring B at 2′ position. The driving force for the elimination of the 2′ hydroxyl to give the ion at *m/e* 253 (M-17) could be provided by the resonance stabilization of the resulting furan structure. UV spectrum [λ_{max}^{MeOH} (log ε): 260 (4.65), 325 (3.81); +$AlCl_3$, 270, 370; +$AlCl_3$–HCl, 270–75; 375; +NaOAc, 270–75, 375 nm] of the aglucone suggested the two hydroxyls in ring A to be at 5 and 7 positions. The third hydroxyl in ring B could only be at position 2′ and thus the structure 5,7,2′-trihydroxyisoflavone (4) has been assigned to the aglucone. A comparison of the UV spectra of the isomeric 5,7,3′- and 5,7,4′-trihydroxyflavones [4, 5] also favours the proposed structure. This is the first report of its natural

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