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ISOFLAVONOIDS FROM *PHASEOLUS COCCINEUS*

S. A. ADESANYA, MELANIE J. O'NEILL and MARGARET F. ROBERTS

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1, U.K.

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Key Word Index—*Phaseolus coccineus*; Leguminosae; isoflavonoid; 7,2',4'-trihydroxy-5-methoxyisoflavone; 7,4'-dihydroxy-5,2'-dimethoxyisoflavanone.

Abstract—After treatment with $CuCl_2$, the following isoflavonoids have been isolated from the runner bean, *Phaseolus coccineus*: daidzein, genistein, isoprunetin, 2'-hydroxygenistein, phaseoluteone, 2'-hydroxydihydrodaidzein, isoferreirin, kievitone, cyclokievitone, glycinol, phaseollidin, phaseollin, demethylvestitol, phaseollinisoflavan, 2'-hydroxyisoprunetin and 7,4'-dihydroxy-5,2'-dimethoxyisoflavanone. The latter two compounds are novel natural products.

INTRODUCTION

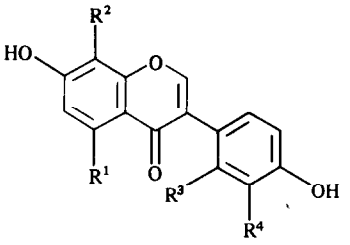
Phaseolus coccineus, the 'runner bean' is grown as a food in Europe, Central America and Africa. In North America it is cultivated mainly as an ornamental climber [1]. In our continuing studies on fungitoxic substances produced by *Phaseolus* species we have investigated the isoflavonoids produced by *P. coccineus* following stress. In an earlier communication [2] we related the isolation of three coumestans viz coumestrol (**21**), aureol (**15**) and isosojagol (**22**) from the runner bean. The present report describes the extraction for the first time of 16 additional isoflavonoids including two novel structures from this plant.

RESULTS AND DISCUSSION

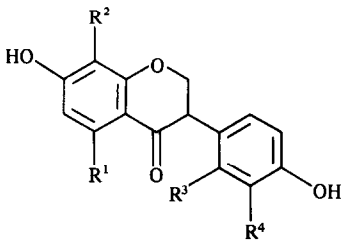
Ethyl acetate extracts from $CuCl_2$-treated *P. coccineus* seedlings were found to contain several fungitoxic substances in a TLC-bioassay against *Cladosporium cucumerinum*. These were not detected in corresponding extracts from control seedlings. Purification of the fungitoxic extract over polyamide and silica gel rendered 16 isoflavonoids (Table 1) which were characterized by spectroscopic methods. In common with several other papilionate legumes [3], *P. coccineus* synthesizes genistein (**2**), 2'-hydroxygenistein (**4**), kievitone (**10**) and demethylvestitol (**16**) after $CuCl_2$ treatment and like some other *Phaseolus* species [3–5] it also produces the more restricted isoflavonoids daidzein (**1**), phaseoluteone (**6**), 2'-hydroxydihydrodaidzein (**7**), isoferreirin (**8**), cyclokievitone (**11**), glycinol (**12**), phaseollidin (**13**), phaseollin (**14**) and phaseollinisoflavan (**17**). The isoflavone isoprunetin (**3**), which we also detected in *P. coccineus*, has not yet been isolated from any other *Phaseolus* species, but has been reported to occur in several other legume genera. *P. coccineus* additionally produced two novel

Table 1. Chromatographic properties and yields of *P. coccineus* isoflavonoids

Compound	Polyamide column fraction no.	TLC solvent (R_f) A	B	C	D	Yield (μg/g fr. wt)
Daidzein (**1**)	2	—	0.53	0.75	—	0.14
Genistein (**2**)	4	—	0.59	0.89	—	0.99
Isoprunetin (**3**)	2	—	0.11	0.40	—	0.07
2'-Hydroxygenistein (**4**)	8	—	0.25	0.40	—	0.61
2'-Hydroxyisoprunetin (**5**)	3	—	0.19	—	—	0.11
Phaseoluteone (**6**)	4	—	0.69	0.70	—	0.32
2'-Hydroxydihydrodaidzein (**7**)	7	—	0.45	0.55	—	0.22
Isoferreirin (**8**)	2	—	0.55	—	0.44	0.14
7,4'-Dihydroxy-5,2'-dimethoxyisoflavanone (**9**)	2	—	0.41	0.99	—	0.13
Kievitone (**10**)	6	—	0.65	0.60	—	1.10
Cyclokievitone (**11**)	5	—	0.50	0.68	—	0.12
Glycinol (**12**)	3	—	0.62	0.59	—	0.05
Phaseollidin (**13**)	2	0.39	0.80	—	—	0.24
Phaseollin (**14**)	1	—	0.90	—	0.89	1.72
Demethylvestitol (**16**)	8	—	0.42	0.58	—	0.61
Phaseollinisoflavan (**17**)	2	0.45	0.80	—	—	0.30



1 $R^1 = R^2 = R^3 = R^4 = H$
2 $R^1 = OH, R^2 = R^3 = R^4 = H$
3 $R^1 = OMe, R^2 = R^3 = R^4 = H$
4 $R^1 = R^3 = OH, R^2 = R^4 = H$
5 $R^1 = OMe, R^3 = OH, R^2 = R^4 = H$
6 $R^1 = R^3 = OH, R^2 = H, R^4 = CH_2CH = CMe_2$
20 $R^1 = R^3 = OH, R^2 = CH_2CH = CMe_2, R^4 = H$



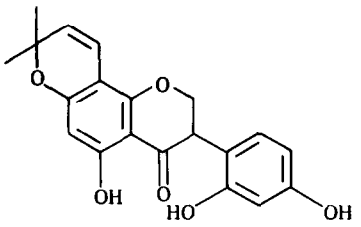
7 $R^1 = R^2 = R^4 = H, R^3 = OH$
8 $R^1 = OH, R^2 = R^4 = H, R^3 = OMe$
9 $R^1 = R^3 = OMe, R^2 = R^4 = H$
10 $R^1 = R^3 = OH, R^2 = CH_2CH = CMe_2, R^4 = H$
18 $R^1 = R^4 = OMe, R^2 = R^3 = H$
19 $R^1 = R^3 = OH, R^2 = R^4 = H$

isoflavonoids: one isoflavone and one isoflavanone.

The first of the new isoflavonoids was provisionally identified as an isoflavone from its UV spectrum in EtOH which possessed a principal maximum at 261 nm. A large bathochromic shift in the spectrum produced upon addition of sodium acetate and a further shift caused by sodium methoxide indicated a C-7 hydroxyl and one or more extra phenolic moieties [7]. Furthermore the shift observed in the aluminium chloride–ethanol spectrum suggested a free hydroxyl either at C-5 or C-2′ [7, 8]. Confirmation of the isoflavone skeleton was provided by the ^{1}H NMR spectrum which contained the characteristic singlet at δ8.17 due to the C-2 proton. A 3H singlet at δ3.90 indicated a methoxyl group and signals between δ6.41 and 7.04 integrated for five aromatic protons. Two of these protons resonate at δ6.51 and 6.53 and show *meta* coupling whilst the three remaining aromatic signals at δ6.41, 6.44 and 7.05 represent a typical ABX system. MS revealed a plausible $[M]^+$ at *m/z* 380 which is the base peak of the spectrum. RDA fragment ions at *m/z* 167 (90%) and *m/z* 134 (9%) are attributable respectively to an A-ring fragment bearing both a hydroxyl and a methoxyl substituent and a B-ring fragment possessing two hydroxyl groups. Thus in the ^{1}H NMR spectrum the signals at δ6.51 and 6.53 which display *meta* coupling must be due to protons at C-6 and C-8 whereas the ABX system of signals represent B-ring protons. The location of the methoxyl at C-5 and a hydroxyl at C-7, which is implied by the UV characteristics was confirmed in a nuclear Overhauser experiment in which irradiation at δ3.90 (methoxyl) produced enhancement of the signal at δ6.51 (C-6) only. The two hydroxyl groups on the B-ring could be located either at C-2′ and C-4′ or at C-2′ and C-5′ to account for the ABX pattern of signals. C-3′ and C-4′ dihydroxylation can be discounted because of the absence of a large shift in the UV spectrum upon addition of aluminium chloride. Since all known legume isoflavonoids are substituted at C-4′ (or the equivalent C-9 position) [3], the location of the hydroxyl groups at C-2′ and C-4′ was favoured. This was confirmed by the fact that the compound gave a positive reaction with Gibbs reagent. Thus the new isoflavone is 7,2′,4′-trihydroxy-5-methoxyisoflavone (2′-hydroxyisoprunetin), **5**.

The second new isoflavonoid possessed a UV absorption maximum at 286 nm which underwent the large bathochromic shifts in sodium acetate and sodium methoxide consistent with the behaviour of isoflavanones having a C-7 hydroxyl and one or more extra phenolic groups in the molecule. The absence of a shift in the maximum in aluminium chloride indicated the lack of a C-5 hydroxyl. The ^{1}H NMR spectrum exhibited characteristic signals for the heterocyclic protons of an isoflavanone at δ4.47 (C-2a), 4.38 (C-2b) and 4.06 (C-3). Signals were observed for five aromatic protons, two of which resonate at δ6.15 and 6.02 and are *meta* coupled and the three remaining protons form an ABX system with signals at δ6.86, 6.49 and 6.35. Two 3H singlets at δ3.79 and 3.75 were assigned to two methoxyl groups. MS revealed a possible $[M]^+$ at *m/z* 316 and important ions at *m/z* 167 and 150 which represent respectively RDA A and B ring fragments each of which possesses one hydroxyl and one methoxyl group. The location of the A-ring methoxyl substituent at C-5 was confirmed by a nuclear Overhauser experiment in which irradiation at δ3.79 produced enhancement of the signal at δ6.15 (C-6). A second NOE involving irradiation at the δ3.75 methoxyl which caused enhancement at only the *meta* coupled signal at δ6.49 and the observation that the compound gave a negative reaction with Gibbs reagent implies that the hydroxyl in the B ring is located at C-4′ whilst the methoxyl resided at either C-2′ or C-5′. Thus two structures: 7,4′-dihydroxy-5,2′-dimethoxyisoflavanone (**9**) and 7,4′-dihydroxy-5,3′-dimethoxyisoflavanone (**18**) can account for the spectroscopic behaviour of the new compound. Considering that several 2′-hydroxylated and 2′-methoxylated isoflavones (**4**–**6**) and isoflavanones (**7**, **8**, **10**, **11**) have been isolated from the plant whereas no 3′-oxygenated derivative has been detected, it seems likely that the new isoflavanone is 7,4′-dihydroxy-5,2′-dimethoxyisoflavanone (**9**).

Plausible biosynthetic interrelationships between isoflavonoids in *Phaseolus* species have been proposed [9–11] and discussed at length earlier [4, 5]. Similar metabolic grids can accommodate the occurrence of 2′-hydroxyisoprunetin (**4**) and 7,4′-dihydroxy-5,2′-dimethoxyisoflavanone (**9**) in *P. coccineus*. 2′-Hydroxyisoprunetin (**4**) is possibly derived from genistein either by 2′-hydroxylation followed by 5-*O*-methylation (i.e. via 2′-hydroxygenistein, **4**) or by 5-*O*-methylation followed by 2′-hydroxylation (i.e. via isoprunetin, **3**). 7,4′-Dihydroxy-5,2′-dimethoxyisoflavanone (**9**) presumably arises by 5-*O*-methylation of isoferreirin (**8**). It is of interest to note that dalbergioidin (**19**), which appears to be a key intermediate



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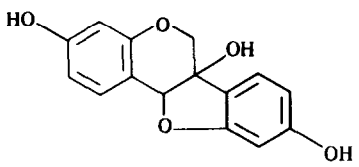

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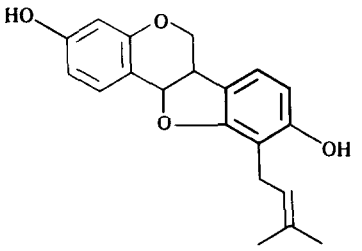

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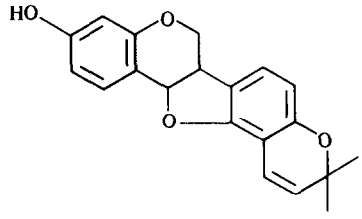

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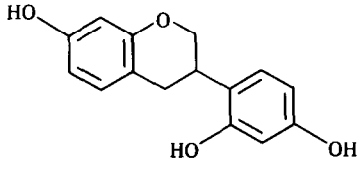

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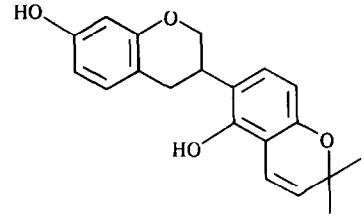

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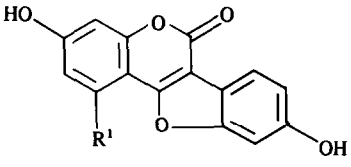
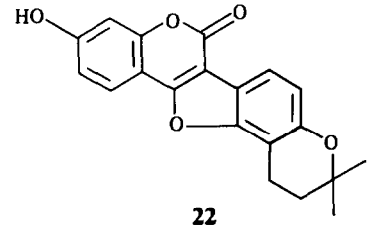


15 R^1 = OH
21 R^1 = H



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in the biosynthesis of kievitone in *P. vularis* could not be detected in *P. coccineus*. Moreover we could not detect 2,3-dehydrokievitone (**20**) which would have provided a possible alternative route to kievitone from 2'-hydroxygenistein avoiding dalbergioidin. It is possible that the apparent lack of dalbergioidin reflects a very high turnover rate for this compound which may also intervene in the biogenesis of isoferreirin (**8**), 7,4'-dihydroxy-5,2'-dimethoxyisoflavanone (**9**) and aureol (**15**) in *P. coccineus*. *Phaseolus coccineus* resembles most *Phaseolus* species examined to date in producing phaseollin as a major isoflavonoid following stress, but it behaves like *P. mungo* (syn *Vigna mungo*) and other *Vigna* species in its ability to produce methoxylated isoflavonoid derivatives.

EXPERIMENTAL

Plant material and extraction of isoflavonoids. Seeds of *P. coccineus* var. scarlet runner were germinated, treated with aq. $CuCl_2$ and extracted as previously described [2]. Column chromatography of the EtOAc extract over polyamide yielded eight fractions which were further purified by TLC on silica gel GF_{254} using the following solvents: hexane–Me_2CO (2:1; solvent A), hexane–EtOAc–MeOH (6:4:1; solvent B), $CHCl_3$–*iso*-

PrOH (9:1; solvent C) and $CHCl_3$–MeOH (10:1; solvent D). The distribution of isoflavonoids in the column fractions and their R_f values in TLC are given in Table 1. Compounds **1**, **2**, **4**, **6–8** and **10–17** were characterized by comparison of their spectroscopic properties with those of authentic standards and literature values [4, 5, 9, 12–16].

Isoprunetin (**3**). UV λ_{max}^{EtOH} nm: 207, 256, 285 sh, 315 sh; EtOH + NaOAc: 266; EtOH + NaOMe; 205, 266: EtOH + $AlCl_3$: 207, 256; MS *m/z* (rel. int.): 284 (9) $[M]^+$, 267 (5) $[M-OH]^+$, 167 (100), 118 (4); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ7.93 (1H, *s*, C-2), 7.39 (2H, *dd*, *J* = 8.0, 1.9 Hz, C-2′, C-6), 6.85 (2H, *dd*, *J* = 7.7, 2.1 Hz, C-3′, C-5′), 6.45 (2H, *s*, C-6, C-8), 3.85 (3H, *s*, C-5 OMe).

2′-Hydroxyisoprunetin (**5**). UV λ_{max}^{EtOH} nm: 206, 256, 290; EtOH + NaOAc: 269, 320; EtOH + NaOMe: 210, 270 sh, 273, 320; EtOH + $AlCl_3$: 206, 263 sh, 268, 293; MS *m/z* (rel. int.): 300 (100) $[M]^+$, 283 (7) $[M-OH]^+$, 167 (90), 134 (9); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ8.04 (1H, *s*, C-2), 7.05 (1H, *d*, *J* = 8.2 Hz, C-6′), 6.53 (1H, *d*, *J* = 2.2 Hz, C-8), 6.51 (1H, *d*, *J* = 2.2 Hz, C-6), 6.44 (1H, *d*, *J* = 2.4 Hz, C-3′), 6.41 (1H, *dd*, *J* = 8.2, 2.4 Hz, C-5′), 3.90 (3H, *s*, C-5 OMe); NOE: irradiation at δ3.90 (C-5 OMe) produced enhancement at δ6.51 (C-6).

7,4′-Dihydroxy-5,2′-dimethoxyisoflavanone (**9**). UV λ_{max}^{EtOH} nm: 205, 229 sh, 286: EtOH + NaOAc: 250 sh, 322; EtOH + NaOMe: 206, 245, 322: EtOH + $AlCl_3$: 229 sh, 286; MS *m/z* (rel. int.): 316 (10) $[M]^+$, 298 (4) $[M-H_2O]^+$, 285 (2), $[M-OMe]^+$, 167 (100), 150 (36); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ6.86 (1H, *d*, *J* = 8.3 Hz, C-6′), 6.49 (1H, *d*, *J* = 2.5 Hz, C-3′), 6.35 (1H, *dd*, *J* = 8.3, 2.5 Hz, C-5′), 6.15 (1H, *d*, *J* = 2.2 Hz, C-6), 6.02 (1H, *d*, *J* = 2.2 Hz, C-8), 4.47 (1H, *t*, *J* = 10.0 Hz, C-2a), 4.38 (1H, *dd*, *J* = 10.1, 5.3 Hz, C-2b), 4.06 (1H, *dd*, *J* = 9.8, 5.3 Hz, C-3), 3.79 (3H, *s*, C-5 OMe), 3.75 (3H, *s*, C-2′ OMe); NOE: irradiation at δ3.75 (C-2′ OMe) produced enhancement at δ6.49 (C-3′); irradiation at δ3.79 (C-5 OMe) produced enhancement at δ6.15 (C-6).

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