Isoflavonoids from the Root Bark of *Piscidia erythrina* and a Note on the Structure of Piscidone

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A methanolic extract of *Piscidia erythrina* root bark has been found to contain various isoflavonoids including rotenone (rotenoid), lisetin (coumaronochromone) and six known isoflavones (ichthynone, piscidone, piscerythrone, 2′-deoxypiscerythrone, 6′-prenylpiscerythrone and 3′,5′-diprenylgenistein). The extract additionally yielded three new 5-hydroxyisoflavones (piscerythrinetin, 2′-hydroxypiscerythrinetin and isowighteone) and a previously unreported coumaronochromone (8-prenyl-lisetin). All four compounds were identified using a combination of spectroscopic (UV, MS, ^{1}H NMR) and chemical methods. Although several other 5-hydroxyisoflavones were also isolated from the root bark extract, the quantities of each were sufficient only to permit their partial characterization. Structure **2** for piscidone has been confirmed by ^{1}H NMR spectroscopy.

Introduction

The roots and root bark of the Jamaican dogwood [*Piscidia erythrina* L. = *P. piscipula* (L.) Sarg.] are known to be rich in isoflavonoid components [1–8], at least two of which (rotenone and ichthynone) contribute towards the fish-poisoning properties associated with this tropical leguminous tree [9]. Thus, earlier studies revealed the presence of seven isoflavones (jamaicin, ichthynone (**1**), piscidone (**2**), piscerythrone (**3**), 2′-deoxypiscerythrone (**4**), 6′-prenylpiscerythrone (**5**), and 3′,5′-diprenylgenistein (**6**)) in addition to rotenone (**7**) and five related rotenoids (deguelin, sumatrol, millettone, isomillettone and dehydromillettone) and the coumaronochromone lisetin (**8**). All the isoflavonoids so far obtained from *P. erythrina* have proved to be 'complex' derivatives containing prenyl (3,3-dimethylallyl), 2,2-dimethylpyran or isopropenyldihydrofuran substituents.

We have recently carried out a detailed phytochemical examination of *P. erythrina* root bark collected during 1987 in the Yucatan region of Mexico. A methanolic extract of this material has yielded, after column and thin-layer chromatography, three new 5-hydroxyisoflavones (**9**–**11**) and a second coumaronochromone (**12**), the structures of which are the subject of this report. Several other isoflavone-like compounds (which could not be fully identified because of a lack of material) were also isolated together with varying quantities of isoflavonoids **1**–**8** previously recognized in *P. erythrina*.

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Results and Discussion

Finely ground *Piscidia* root bark (500 g) was exhaustively extracted with 90% aqueous methanol (4 × 4.5 l) over a 20 day period. The combined extracts were then concentrated and worked up as described in the Experimental section to afford a final ethyl acetate solution which upon chilling deposited crude piscidone (**2**). This material was collected by filtration, and recrystallized from acetone to yield approx. 2.8 g of pure **2**. The filtrate and mother liquor were subsequently fractionated by column chromatography (see Experimental section for details) using mixtures of ethyl acetate and benzene as the principal eluting solvent. Each column fraction (denoted Fr) was concentrated, chilled (−10 °C) to encourage deposition of crystals, and examined by preparative Si gel TLC in various solvent systems.

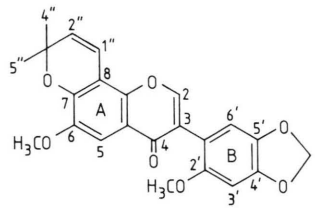
Apart from rotenone (**7**) (1.5 g), column fraction (Fr)-7 afforded two new isoflavonoids (PE-1,

3.4 mg, and PE-2, 3.8 mg) and the known isoflavone 3′,5′-diprenylgenistein (**6**) (11.7 mg) [8]. Fraction 8 proved to be especially rich in isoflavonoid constituents, yielding a quantity of rotenone (0.8 g), the coumaronochromone lisetin (**8**) (4.4 mg) [1, 5] and two known isoflavones, piscerythrone (**3**) (9.6 mg) [5] and 2′-deoxypiscerythrone (**4**) (77 mg) [6]. In addition to these compounds, Fr-8 also contained a further amount of PE-1 (12.6 mg), first detected in Fr-7, and six new isoflavones denoted PE-3 (60 mg), PE-4 (14 mg), PE-5 (88 mg), PE-6 (6 mg), PE-7 (13.2 mg) and PE-8 (8 mg). Column fraction 9 (**3** and 6′-prenylpiscerythrone (**5**)), Fr-10 (ichthynone (**1**), plus **3** and **5**), Fr-11 (**1** and **5**), Fr-12 to Fr-14 (**1** and piscidone (**2**)) and Fr-15 (**2**) contained only known isoflavones [4–6]. No compounds of interest were detected in the early column fractions Fr-1 to Fr-6. Although the amounts were not measured precisely, our studies clearly show that the Mexican *Piscidia* root bark (500 g) contains piscidone (5 g), piscerythrone (4 g), 6′-prenylpiscerythrone (4 g), ichthynone (3 g) and rotenone (2.5 g) as major isoflavonoid components, with the remaining compounds occurring in very much smaller quantities (5–100 mg).

As noted above, the *Piscidia* extract yielded eight new isoflavonoids referred to by an appropriate PE number. Of these, however, only PE-2 (a coumaronochromone), PE-3, PE-5 and PE-8 (all isoflavones) could be fully identified, although spectroscopic (UV, MS, ^{1}H NMR) data for the remaining four compounds (PE-1, -4, -6, and -7) are included in the Experimental section.

The ^{1}H NMR spectrum of PE-3 (M^+ 382) revealed that rings A and C were substituted as in genistein (5,7,4′-trihydroxyisoflavone). Dihydroxylation of ring A was evident from the MS ion at *m/z* 153 (**18**), the OH groups being readily assigned to C-5 (bathochromic UV shift of the MeOH maximum at 261 nm upon addition of $AlCl_3$ [10], and detection of a very low field ^{1}H NMR singlet at δ 13.02 [11]) and C-7 (bathochromic UV shift caused by addition of NaOAc [10]). Attention was therefore concentrated on ring B where two *meta*-coupled protons were accompanied by a prenyl sidechain and a pair of methoxyl substituents.

Assuming oxygenation (OCH_3) at C-4′ as in all other recognized *Piscidia* isoflavones [3–8], only the relative positions of the second OCH_3 group and the prenyl substituent remained to be determined. The



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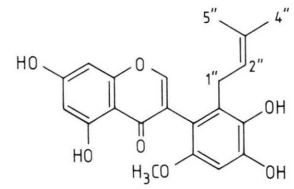

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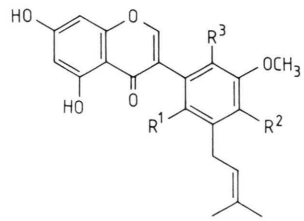
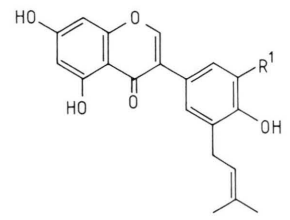


3: $R^1 = R^2 = OH$; $R^3 = H$
4: $R^1 = R^3 = H$; $R^2 = OH$
5: $R^1 = R^2 = OH$; R^3 = Prenyl
9: $R^1 = R^3 = H$; $R^2 = OCH_3$
10: $R^1 = OH$; $R^2 = OCH_3$; $R^3 = H$



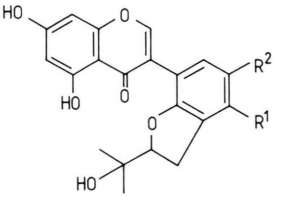
6: R^1 = Prenyl
11: $R^1 = H$

B-ring protons of PE-3 exhibited chemical shift values similar to those reported for H-2′ and H-6′ of 2′-deoxypiscerythrone (**4**) [6] and this, together with the appearance of two distinct methoxyl signals (δ 3.81 and 3.89) indicative of an asymmetric disposition, allowed ring B to be formulated as in **9**. We believe, however, that the B-ring proton assignments (H-2′ = δ 7.10, and H-6′ = δ 6.95) made by Delle Monache *et al.* [6] for 2′-deoxypiscerythrone (**4**) should be reversed, and this view is supported by our unpublished work involving substituent effects on the ^{1}H NMR signals given by various model compounds. Thus, in **9** the signals at δ 7.02 and 7.15 were assigned to H-2′ and H-6′ respectively. Corresponding values for 2′-deoxypiscerythrone would therefore be δ 6.95 (H-2′) and 7.10 (H-6′).

Although it is not pertinent to discuss in detail the substituent effect studies mentioned above, it should be notet that PE-3 (**9**) and 2′-deoxypiscerythrone differ only in the possession, by the former compound, of a 4′-OCH_3 substituent. We have found that 4′-O-methylation of a 4′-hydroxy (genistein-type) isoflavone shifts the B-ring *o*-(3′/5′) and *m*-(2′/6′) proton signals by + 0.05 ppm (*o*, δ 6.90 → 6.95; *m*, δ 7.45 → 7.50). Methylation of 2′-deoxypiscerythrone at C-4′ would therefore be expected to shift the revised *meta* (H-2′/H-6′) signals to δ 7.00 and 7.15 respectively, values in very good agreement with those assigned from actual measurements to H-2′ (δ 7.02) and H-6′ (δ 7.15) of **9**. We suggest that **9** should be named piscerythrinetin.

The MS of PE-5 (M^+ 398) immediately suggested that this compound was a hydroxy derivative of piscerythrinetin (**9**). UV, MS and ^{1}H NMR data confirmed that both compounds had identical A-rings (5,7-dihydroxylated), and that ring B of PE-5 possessed a prenyl substituent and two methoxyl groups. Moreover, only one proton signal (δ 6.83s) was assignable to ring B indicating the presence of a fourth substituent which, from the MS fragment at *m/z* 191 (**19**), was considered to be OH.

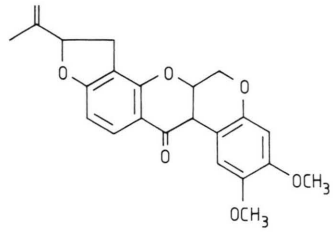
The location of this OH group at C-2′ was initially inferred from a comparison of the position of the 5-O<u>H</u> signal of PE-5 with that of licoisoflavone A (**13**; 5,7-dihydroxy substitution only on ring A) and its pyrano and furano derivatives having either a 3′ → 2′ [0] or 3′ → 4′ [0] side structure disposition, *e.g.* **14** and **15** [12]. In the model **13** and its cyclo derivatives with 2′-hydroxylation, the 5-O<u>H</u> signal appeared between δ 12.54 and 12.67 whereas in cyclic analogues with the 2′-OH derivatized the corresponding signal was shifted to δ 13.02–13.14. Detection of the 5-O<u>H</u> signal of PE-5 at δ 12.53 is therefore in accord with the occurrence of a 2′-hydroxyl group*. In addition, treatment of PE-5 with *m*-chloroperoxybenzoic acid (*m*-CPBA) as described in the Experimental section afforded predominantly the dihydrofurano-isoflavone **16** with the 5-O<u>H</u> signal at δ 12.99. Apart from supporting the above NMR discussion, the formation of **16** established that the prenyl substituent was adjacent to the OH group at C-2′.



15: R^1 = OH ; R^2 = H
16: R^1 = R^2 = OCH_3

Again, if oxygenation at C-4′ is assumed, the remaining B-ring OCH_3 group of PE-5 must be attached at either C-5′ (as in **9**) or C-6′. The slow (genistein-type) reaction of PE-5 when subjected to the Gibbs test [13, 14] provided evidence for substitution (5′) *ortho* to the 2′-OH group (a very rapid reaction would have been expected in the absence of 5′-substitution). Additionally, the B-ring proton of dihydrofurano-isoflavone **16** (derived by *m*-CPBA cyclization of PE-5) had a chemical shift value (δ 7.12) comparable with that assigned by us to H-6′ of **9** (see earlier discussion). A ^{1}H NMR spectrum of piscerythrone (**3**) obtained from Mexican *Piscidia* root afforded H-6′ at δ 6.77 (*cf.* Ref. [6], H-6′ = δ 6.72 in the same solvent [acetone-d_6]). Allowing for a substituent effect of + 0.05 ppm upon the *meta* pro-

* Although piscidone is frequently represented by **2**, to our knowledge no data have yet been published to firmly exclude the alternative structure [5] with 2′-OH and 4′-OCH_3 substituents. The ^{1}H NMR spectrum of pure piscidone determined in acetone-d_6 revealed the 5-O<u>H</u> signal at δ 13.01, a result which, from the foregoing discussion, indicates the absence of a 2′-OH group. Since the B-ring substituents at C-3′ (H), C-5′ (OH) and C-6′ (prenyl) have already been established [5], our spectroscopic studies confirm the structure of piscidone as **2** (2′-OCH_3, 4′-OH) without the need to resort to a lengthy chemical investigation.



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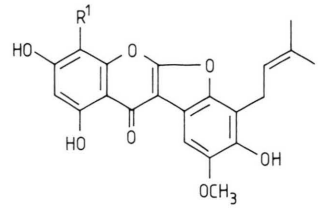
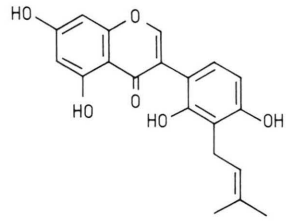


8: R^1 = H
12: R^1 = Prenyl



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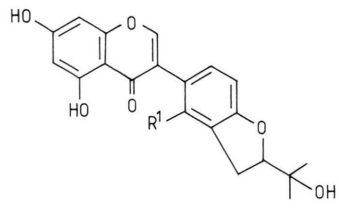


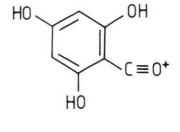
14: R^1 = OH
17: R^1 = H

ton (H-6′) following 4′-methylation of **3**, the theoretical chemical shift value (δ 6.82) for H-6′ essentially coincides with that observed in the NMR spectrum of PE-5 (H-6′, δ 6.83). The above evidence permits PE-5 to be assigned structure **10** for which we propose the common name 2′-hydroxypiscerythrinetin (4′-O-methylpiscerythrone).

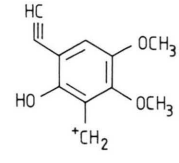
The identity of *Piscidia* isoflavone PE-8 as the genistein derivative **11** was readily established by spectroscopic and chemical studies. Ring A was found to be substituted only at C-5 (OH) and C-7 (OH), whilst ^{1}H NMR chemical shift values for the three B-ring protons closely resembled those reported for H-2′, -5′ and -6′ of the known *Lupinus* isoflavone lupalbigenin with 3′ (prenyl) and 4′ (OH) substitution [15]. PE-8 was also found to be prenylated on ring B, with an OH group being assigned to C-4′ in order to fully satisfy the MS (M^+ 338; B-ring ion *m/z* 131, **20**) and ^{1}H NMR data. Upon treatment with *m*-CPBA, PE-8 afforded a cyclo derivative (**17**) indistinguishable (UV, MS, ^{1}H NMR) from lupinisoflavone C [14]. Thus, the prenyl substituent must be located *ortho* to the 4′-OH group as shown in **11**. The common name isowighteone (3′-prenylgenistein) is suggested for this previously unrecognized compound.

The fourth *Piscidia* isoflavonoid (PE-2, M^+ 450) was identified as a coumaronochromone from its distinct UV (MeOH) spectrum, and the lack of a low field ^{1}H NMR singlet attributable to H-2 [16]. Apart from dihydroxylation (C-5 and C-7), ring A was found to possess a prenyl substituent, the remaining proton appearing as a singlet at δ 6.44. Ring B similarly contained a prenyl sidechain and was formulated as in **12** from a ^{1}H NMR comparison with lisetin (**8**), a known *Piscidia* coumaronochromone [5] reisolated during the course of the present study. Both lisetin (**8**) and **12** afforded virtually coincident B-ring ^{1}H NMR signals.

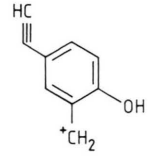
Assignment of the A-ring prenyl substituent to C-8 rather than to C-6 was based on two lines of evidence. First, PE-8 did not afford a characteristic blue



18: *m/z* 153



19: *m/z* 191



20: *m/z* 131

or blue-green Gibbs test colour, a result consistent with substitution *para* to the C-5 hydroxyl group [13, 14]. Secondly, in the ^{1}H NMR spectrum of PE-8 the single A-ring proton appeared at a position (δ 6.44) similar to that noted earlier in acetone-d_6 for H-6 of the model coumaronochromone lupilutin (δ 6.42) [17]. In contrast, the A-ring proton (H-8) of lupinalbin B (OH at C-5 and C-7; prenyl at C-6) occurs at significantly lower field (δ 6.66 [16]) thereby permitting PE-8 to be assigned structure **12**. We suggest that **12** should be named 8-prenyl-lisetin to indicate its relationship to **8**.

The remaining *Piscidia* compounds (PE-1, -4, -6 and -7), which we name piscidisoflavones A–D respectively, were all found to be 5-hydroxy isoflavones although none could be fully characterized owing to the small quantity of material (5–15 mg) available for examination. However, spectroscopic data for piscidisoflavones A–D are included in the Experimental section, whilst Table I summarizes information on the nature and, where possible, location of the various A- und B-ring substituents.

It has previously been noted that samples of *P. erythrina* root bark differ in terms of their isoflavonoid composition [4, 6]. Thus, in addition to the isoflavones jamaicin, ichthynone, piscidone and piscerythrone, and the coumaronochromone lisetin, Ollis and his co-workers [4, 5] isolated rotenone (rotenoid), its 5-hydroxy analogue (sumatrol) and three new 2,2-dimethylpyran substituted rotenoids (millettone, isomillettone and dehydromillettone) from *Piscidia* root bark of Jamaican origin. More recent studies [6, 8] failed to reveal either sumatrol or ichthynone but yielded the rotenoid deguelin and a number of new isoflavones (2′-deoxypiscerythrone, 6′-prenylpiscerythrone and 3′,5′-diprenylgenistein).

Our own detailed investigation of *Piscidia* root bark collected in Mexico tends to confirm the chemical variability noted by other workers. For example, we were unable to identify any of the known *Piscidia* rotenoids apart from rotenone, although two new rotenoids each with an ichthynone-type A-ring were detected. These compounds will be described in a separate paper. Jamaicin, a major component of Jamaican *Piscidia* root bark [5] was not obtained from our material, whereas ichthynone (isolated in abundance by Ollis *et al.* [4, 5] but not at all by Delle Monache *et al.* [6]) was extracted in large quantities.

Lisetin, readily isolated from Jamaican *Piscidia* root [5, 6], was barely detectable in our sample of

Table I. Summary of molecular weights, and A- and B-ring substituents of piscidisoflavones A–D.

Compound	Mol. wt.	Substituents[a,b] Ring A	Ring B
Piscidisoflavone A[c] (PE-1)	452	OH(5), H(6), OH(7), Pr(8)	2× OH, OCH_3, Pr(3′)*, H(6′)*
Piscidisoflavone B (PE-4)	450	OH(5), H(6), OH(7), H(8)	OCH_3(2′)*, Py(3′→4′[0])*, OH(5′)*, Pr(6′)*
Piscidisoflavone C (PE-6)	382	OH(5), H(6)*, Py(8→7[0])*	2× OH, OCH_3, H(3′)*, H(6′)*
Piscidisoflavone D (PE-7)	382	OH(5), H(6), OH(7), H(8)	OCH_3(2′)*, Py(3′→4′[0])*, OH(5′)*, H(6′)*

[a] OH = hydroxyl; OCH_3 = methoxyl; Pr = prenyl (3,3-dimethylallyl); Py = 2,2-dimethylpyran; H = proton. The position of a substituent on ring A or ring B is shown by the carbon number in parentheses. A provisional location is indicated by an asterisk (*).

[b] The assignment of a prenyl substituent to either C-3′ (piscerythrone type) or C-6′ (piscidone type) is based on a ^{1}H NMR comparison of the side chain methyl signals of piscidisoflavone A and B with those of **2** (piscidone) and **3** (piscerythrone). In **2**, these signals appear at unusually high field (δ 1.43 and 1.51 in acetone-d_6 [19]; *cf.* piscidisoflavone B, δ 1.40 and 1.50) whereas in **3** they occur at δ 1.65 and 1.78 [19]; *cf.* piscidisoflavone A, δ 1.67 and 1.81. Other provisional assignments reflect the Gibbs test response, and general comparisons with known isoflavonoids, particularly those from *P. erythrina*.

[c] Piscidisoflavone A may possibly be 8-prenylpiscerythrone, the assumed precursor of 8-prenyl-lisetin (**12**).

Mexican origin. Although variations in the isoflavonoid content of *Piscidia* samples may be partly due to factors such as conditions of growth, age and amount of material examined etc., the marked differences with respect to major root bark components (notably jamaicin, ichthynone, lisetin, millettone and isomillettone) provide some support for the view [6] that chemical races might exist within the species *P. erythrina*.

Experimental

Root bark of *Piscidia erythrina* L. [*P. piscipula* (L.) Sarg.] was collected during 1987 by Dr. C. E. Hughes (Oxford Forestry Institute) from a tree growing near Tixcacalcupa, Yucatan Province, Mexico. Upon arrival in Reading, the air-dried material was milled to a fine powder and immediately extracted with aqueous MeOH as outlined below. Preparative thin-layer chromatography (PTLC) of root bark isoflavonoids was undertaken on Merck precoated plates (Si gel, F-254, layer thickness 0.5 mm) in one or more of the following solvent systems: BE = benzene–EtOAc (5:1), CAAm = $CHCl_3$–acetone–conc. aqueous NH_3 (35:30:1, 25:50:1 or 10:50:1), CBE = $CHCl_3$–benzene–EtOAc (1:3:1), CM = $CHCl_3$–MeOH (25:1), HEA = *n*-hexane–EtOAc–acetone (5:1:1). ^1H NMR spectra were determined in acetone-d_6 at 100 MHz (TMS reference).

Isolation of Piscidia isoflavonoids

Finely powdered *P. erythrina* root bark (500 g) was extracted with 90% aqueous MeOH (4 × 4.5 l) at room temperature over a period of approx. 20 days. The combined MeOH extracts were then reduced *in vacuo* (35–40 °C) to 300 ml and shaken with EtOAc (4 × 300 ml). This EtOAc extract was washed successively with 5% aqueous $NaHCO_3$ (2 × 250 ml) and brine (3 × 300 ml) before being dried over anhydrous Na_2SO_4, and concentrated to near dryness. Dilution to 50 ml with EtOAc, followed by chilling (0 °C) for several days caused the deposition of impure piscidone (**2**) which was collected by filtration and recrystallized from acetone. The filtrate and the mother liquor remaining after crystallization were combined and reduced to dryness, the resulting solid (35 g) then being redissolved in EtOAc and adsorbed onto Wako-gel (100 g). After removing the solvent, the dry powder was applied to a column of Wako-gel (280 g) settled in benzene (Bz). Isoflavonoids were eluted from the column using mixtures of EtOAc in Bz. The first 300 ml (column void) of eluate (Fraction O, Bz only) were discarded. Thereafter, fraction volumes were mostly 200–300 ml. Fractions 1–21 were eluted using the following solvents or solvent mixtures: Fraction (Fr)-1 and -2 (Bz only, both 300 ml), Fr-3, -4 and -5 (10% EtOAc in Bz, all 200 ml), Fr-6, -7, -8 and -9 (15% EtOAc in Bz, all 200 ml), Fr-10 -11 and -12 (20% EtOAc in Bz, all 200 ml), Fr-13, -14 and -15 (30% EtOAc in Bz, all 200 ml), Fr-16, -17 and -18 (50% EtOAc in Bz, all 200 ml), Fr-19 (EtOAc only, 200 ml), Fr-20 (EtOAc only, 500 ml) and Fr-21 (EtOAc–MeOH, 1:1, 1000 ml). Each eluate was concentrated to between 1 and 10 ml and chilled (−10 °C) to encourage the deposition of crystals or precipitates which were recovered by filtration. Isoflavonoids in the eluates were also isolated and purified by preparative TLC on Si gel.

Fractions 1–6 yielded no major compounds of interest, whilst fractions 9–15 contained varying amounts of the known isoflavones ichthynone (**1**), piscidone (**2**), piscerythrone (**3**) and 6′-prenylpiscerythrone (**5**). These compounds were easily separated by Si gel PTLC in CM, 25:1 (**1**, R_F 0.88; **2**, R_F 0.26; **3**, R_F 0.60; **5**, R_F 0.36).

PTLC of Fr-7 (BE, 5:1) afforded three bands, Fr-7-1 to Fr-7-3, the first (R_F 0.57) being attributable to rotenone (**7**). Band Fr-7-2 contained traces of several compounds but these were not examined in detail. The lowest band (Fr-7-3) was divided into two major fractions (Fr-7-3-1 and Fr-7-3-2) by PTLC in CAAm (35:30:1). Elution (EtOAc) and careful re-PTLC of Fr-7-3-1 in the same solvent gave the coumaronochromone 8-prenyl-lisetin (**12**, R_F 0.58) and the isoflavone piscidisoflavone A (R_F 0.49). Finally, elution and successive re-PTLC of Fr-7-3-2 in HEA (5:1:1) and CM (25:1) yielded pure 3′,5′-diprenylgenistein (**6**). Rotenone (**7**) proved to be the major isoflavonoid component of fraction 7, a total of 1.5 g being isolated from this source.

Upon concentration and chilling, Fr-8 deposited material (1.16 g) consisting mainly of piscerythrone (**3**). This was collected by filtration, the filtrate then being divided into five sub-fractions (Fr-8-1 to Fr-8-5) by PTLC in CBE (1:3:1). The uppermost band (Fr-8-1) afforded only rotenone (**7**), whilst the second (Fr-8-2) gave traces of a rotenoid-like substance

which was not further examined. Elution with EtOAc and re-PTLC of the third band (Fr-8-3) in CM (25:1) yielded piscerythrinetin (**9**). Sub-fraction Fr-8-4 was separated into four isoflavonoid-rich bands (Fr-8-4-1 to Fr-8-4-4) by PTLC in CAAm (25:50:1), the uppermost of these being identical with piscidisoflavone A from Fr-7. The remaining three bands were further chromatographed in HEA (5:1:1) to give the following compounds: Fr-8-4-2 → piscidisoflavone B; Fr-8-4-3 → lisetin (**8**) (upper zone) and 2′-deoxypiscerythrone (**4**) (lower zone); Fr-8-4-4 → 2′-hydroxypiscerythrinetin (**10**) (upper zone) and piscerythrone (**3**) (lower zone). Finally, the lowest running CBE subfraction (Fr-8-5) of Fr-8 was chromatographed in CAAm (10:50:1) to yield piscidisoflavone C (upper zone), a mixture of piscidisoflavone D and isowighteone (**11**) (middle zone) and more piscerythrone (**3**) (lower zone). Isowighteone (**11**, R_F 0.29) and piscidisoflavone D (R_F 0.46) were eventually separated by PTLC in CM (25:1).

Ichthynone (**1**), *piscidone* (**2**), *piscerythrone* (**3**), *2′-deoxypiscerythrone* (**4**), *6′-prenylpiscerythrone* (**5**), *3′,5′-diprenylgenistein* (**6**), *rotenone* (**7**) *and lisetin* (**8**)

Identification of the above previously reported *Piscidia* compounds was based on direct spectroscopic (UV, MS, ^{1}H NMR) and chromatographic comparisons with authentic material, or by reference to literature values [4–8].

Piscerythrinetin (**9**) *(= PE-3)*

Colourless fine rods, m.p. 177–178 °C. Appearance on Si gel TLC plates viewed under long wavelength (365 nm) UV light, dark purple. Gibbs test response [13, 14], (+), slow, purple. UV: λ_{max}, nm: MeOH 261, 285sh (br), 320sh (br); + NaOMe 273, 330 (br); + $AlCl_3$ 269.5, 302sh, 363; + NaOAc 271, 330 (br) (addition of solid boric acid regenerated the MeOH spectrum [10]). MS (rel. int.): *m/z* 382 (M^+; 100), 381 (21), 367 (25), 351 (12), 327 (M^+ − 55; 5), 326 (4), 325 (13), 175 (B-ring fragment with CH_2 remnant from prenyl sidechain; 4), 153 (dihydroxy A-ring fragment; 66). ^{1}H NMR: δ 1.71s and 1.75s (both 3H, 4″- and 5″-C$\underline{H}_3$), 3.36br d (2H, J=7.0 Hz, 1″-C$\underline{H}_2$), 3.81s and 3.89s (both 3H, 4′- and 5′-OC$\underline{H}_3$), 5.30br t (J=*ca.* 7.0 Hz, 2″-C$\underline{H}$), 6.30d (J=2.4 Hz, H-6), 6.43d (J=2.4 Hz, H-8), 7.02d (J=2.4 Hz, H-2′), 7.15d (J=2.4 Hz, H-6′), 8.23s (H-2), 13.02s (5-O$\underline{H}$).

2′-Hydroxypiscerythrinetin (**10**) *(= PE-5)*

Pale yellow gum. Long wavelength UV fluorescence, dark purple. Gibbs test response, (+), slow, blue. UV: λ_{max}, nm: MeOH 220sh, 262, 297 (br); + NaOMe 274, 300sh (br), 325sh (br); + $AlCl_3$ 225sh, 266, 300sh, 360; + NaOAc 274, 298sh, 330sh (br) (addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 398 (M^+; 100), 383 (6), 356 (6), 355 (M^+ − 43; 23), 344 (11), 343 (M^+ − 55; 53), 342 (58), 328 (16), 327 (38), 299 (27), 203 (12), 191 (B-ring fragment with CH_2 remnant; 12), 153 (dihydroxy A-ring fragment; 24), 147 (10). ^{1}H NMR: δ 1.66s and 1.78s (both 3H, 4″- and 5″-C$\underline{H}_3$), 3.43br d (2H, J=6.8 Hz, 1″-C$\underline{H}_2$), 3.82s (6H, 4′- and 5′-OC$\underline{H}_3$), 5.26br t (J=6.8 Hz, 2″-C$\underline{H}$), 6.36d (J=2.0 Hz, H-6), 6.51d (J=2.0 Hz, H-8), 6.83s (H-6′), 8.31s (H-2), 12.53s (5-O$\underline{H}$).

Oxidation of **10** *with m-chloroperoxybenzoic acid (m-CPBA)*

2′-Hydroxypiscerythrinetin (**10**) (8 mg) and *m*-CPBA (6.5 mg) were dissolved in $CHCl_3$–acetone (1:1, 0.5 ml) and allowed to stand for 24 h at room temperature (15 °C) [18]. Si gel TLC of the reaction mixture in CM (25:1) gave predominantly the dihydrofurano-isoflavone (**16**) (R_F 0.28) together with a small amount of unchanged **10** (R_F 0.55). Physicochemical data recorded for **16** were as follows: pale yellow gum; long wavelength UV fluorescence, dark purple; Gibbs test response, (+), slow purple-blue. MS (rel. int.): *m/z* 414 (M^+; 26), 357 (19), 356 (82), 355 (M^+ − 59; 100), 313 (13), 153 (9), 69 (9), 59 (12). ^{1}H NMR: δ 1.20s and 1.29s (both 3H, 4″- and 5″-C$\underline{H}_3$ attached to a carbinol carbon), 3.28d (2H, J=8.3 Hz, 1″-C$\underline{H}_2$), 3.80s and 3.89s (both 3H, 4′- and 5′-OC$\underline{H}_3$), 4.63t-like (J=8.3 Hz, 2″-C$\underline{H}$), 6.30d (J=2.4 Hz, H-6), 6.44d (J=2.4 Hz, H-8), 7.12s (H-6′), 8.41s (H-2), 12.99s (5-O$\underline{H}$).

Isowighteone **11** *(= PE-8)*

Colourless, amorphous solid. Long wavelength UV fluorescence, dark purple. Gibbs test response, (+), slow, purple-blue. UV: λ_{max}, nm: MeOH 262, 286sh, 325sh (br); + NaOMe 274.5, 322 (br); + $AlCl_3$ 233, 269, 303sh, 362; + NaOAc 270.5, 327 (br)

(addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 338 (M^+; 100), 323 (17), 321 (9), 309 (13), 295 ($M^+ - 43$; 15), 284 (16), 283 ($M^+ - 55$; 89), 282 (16), 255 (8), 254 (12), 253 (24), 154 (13), 153 (dihydroxy A-ring fragment; 59), 152 (8), 141 (8), 131 (B-ring fragment with CH_2 remnant; 8). ^{1}H NMR: δ 1.73s (6H, 4″- and 5″-C$\underline{H}_3$), 3.37br d (2H, J=7.3 Hz, 1″-C$\underline{H}_2$), 5.38br t (J=7.3 Hz, 2″-C$\underline{H}$), 6.29d (J=2.0 Hz, H-6), 6.42d (J=2.0 Hz, H-8), 6.89d (J=7.8 Hz, H-5′), 7.28 (incomplete dd, H-6′), 7.34 (incomplete d, H-2′), 8.14s (H-2), 13.07s (5-O$\underline{H}$).

Oxidation of **11** *with m-CPBA*

Isowighteone (**11**) (1.1 mg) and *m*-CPBA (0.9 mg) were dissolved in $CHCl_3$–acetone (1:1, 0.2 ml) and allowed to stand at room temperature (15 °C) for 16 h [18]. Si gel TLC of the reaction mixture (CM, 25:1) afforded **17** (0.9 mg) indistinguishable by UV, MS and ^{1}H NMR comparison from authentic lupinisoflavone C [14].

8-Prenyl-lisetin (**12**) *(= PE-2)*

Colourless fine needles, m.p. 243–245 °C. Long wavelength UV fluorescence orange-yellow. Gibbs test response, (−), brown. UV: λ_{max}, nm: MeOH (rel. int. [16]): 261 (100), 286 (58), 304sh (37), 345 (35); + NaOMe 280, 306, 320sh; + $AlCl_3$ 222, 266, 278, 291sh, 322sh, 385; + NaOAc 261 (br), 293, 302sh, 353 (addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 450 (M^+; 100), 395 ($M^+ - 55$; 31), 394 (62), 379 (24), 339 (10), 326 (10), 121 (20). ^{1}H NMR: δ 1.69s, 1.87s and 1.89s (6H, 3H and 3H, 4″-, 5″-, 4‴- and 5‴-C$\underline{H}_3$), 3.55br d (2H, J=7.3 Hz, 1‴-C$\underline{H}_2$), 3.63 (2H, J=7.3 Hz, 1″-C$\underline{H}_2$), 3.98s (3H, 3′-OC$\underline{H}_3$), 5.30br t (J=7.3 Hz, 2‴-C$\underline{H}$), 5.39br t (J=7.3 Hz, 2″-C$\underline{H}$), 6.44s (H-6), 7.36s (H-2′), 12.98s (5-O$\underline{H}$).

Piscidisoflavone A (= PE-1)

Colourless gum. Long wavelength UV fluorescence, dark purple. Gibbs test response, (−), yellow → dark brown. UV: λ_{max}, nm: MeOH 270, 298; + NaOMe 230sh, 287, 310sh; + $AlCl_3$ 230sh, 277.5, 303sh, 364 (br); + NaOAc 275, 284sh, 302sh (addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 452 (M^+; 100), 410 (13), 409 ($M^+ - 43$; 50), 398 (10), 397 ($M^+ - 55$; 42), 396 (16), 381 (31), 353 (27), 342 (11), 341 (56), 340 (19), 325 (31), 233 (12), 231 (18), 217 (21), 189 (24), 177 (B-ring fragment with CH_2 remnant; 25), 170 (14), 165 (dihydroxy A-ring fragment with CH_2 remnant; 17), 163 (10), 149 (11). ^{1}H NMR: δ 1.67s and 1.81s (both 6H, 4″-, 5″-, 4‴- and 5‴-C$\underline{H}_3$), 3.46br d and 3.55br d (both 2H, J=8.3 Hz, 1″- and 1‴-C$\underline{H}_2$), 3.81s (3H, OC$\underline{H}_3$), 5.32br t (2H, 2″- and 2‴-C$\underline{H}$), 6.51s (H-6), 6.94s (H-6′), 8.33s (H-2), 13.04s (5-O$\underline{H}$).

Piscidisoflavone B (= PE-4)

Pale yellow semi-solid. Long wavelength UV fluorescence, dark purple. Gibbs test response, (+), slow purple. UV: λ_{max}, nm: MeOH 226, 254sh, 261; + NaOMe 269, 330 (br); + $AlCl_3$ 227sh, 269, 313; + NaOAc 269, 327 (br) (addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 450 (M^+; 63), 436 (30), 435 ($M^+ - 15$; 100), 395 ($M^+ - 55$; 9), 368 (9), 298 (24), 283 (27), 219 (10), 202 (10), 153 (dihydroxy A-ring fragment; 58). ^{1}H NMR: δ 1.40s (9H, prenyl C$\underline{H}_3$, and 2 × C$\underline{H}_3$ of a dimethylpyran unit), 1.51s (prenyl C$\underline{H}_3$), 3.17t-like (2H, J=6.8 Hz, prenyl C$\underline{H}_2$), 3.83s (3H, OC$\underline{H}_3$), 4.95t-like (J=6.8 Hz, prenyl C$\underline{H}$), 5.59d and 6.16d (both J=10.3 Hz, C$\underline{H}$=C$\underline{H}$C of a dimethylpyran unit), 6.29d (J=2.4 Hz, H-6), 6.44d (J=2.4 Hz, H-8), 7.89s (H-2), 12.91s (5-O$\underline{H}$).

Piscidisoflavone C (= PE-6)

Pale yellow gum. Long wavelength UV fluorescence, dark purple. Gibbs test response, probably (−), yellow → green/brown. UV: λ_{max}, nm: MeOH 227, 282, 303sh; + NaOMe 278sh, 288, 303sh; + $AlCl_3$ 224, 280sh, 292sh, 302, 356. The MeOH spectrum was unaffected by addition of NaOAc. MS (rel. int.): *m/z* 382 (M^+; 65), 368 (28), 367 ($M^+ - 15$; 100), 204 (12), 203 (A-ring fragment with OH group and a dimethylpyran unit minus one CH_3; 88), 183 (19), 176 (13), 127 (24). ^{1}H NMR: δ 1.60s (6H, 2 × C$\underline{H}_3$ of a dimethylpyran unit), 3.93s (3H, OC$\underline{H}_3$), 5.92d and 6.81d (both J=10.3 Hz, C$\underline{H}$=C$\underline{H}$C of a dimethylpyran unit), 6.54s (A-ring $\underline{H}$), 6.64s and 7.05s (B-ring, 2 × $\underline{H}$), 8.36s (H-2), 13.31s (5-O$\underline{H}$).

Piscidisoflavone D (= PE-7)

Fine pale brown rods, m.p. 252–254 °C. Long wavelength UV fluorescence, dark purple. Gibbs test response, (+), slow, purple. UV: λ_{max}, nm: MeOH 222sh, 252sh, 260, 286sh, 327 (br); +

NaOMe 270, 329 (br); + $AlCl_3$ 227sh, 270, 312; + NaOAc 269, 332 (br) (addition of boric acid regenerated the MeOH spectrum). MS (rel. int.): *m/z* 382 (M^+; 35), 368 (24), 367 ($M^+ - 15$; 100), 309 (10), 176 (7), 153 (dihydroxy A-ring fragment; 17). ^1H NMR: δ 1.36s (6H, 2 × $C\underline{H}_3$ of a dimethylpyran unit), 3.80s (3H, $OC\underline{H}_3$), 5.72d and 6.73d (both $J = 9.8$ Hz, $C\underline{H}=\underline{H}C$ of a dimethylpyran unit), 6.28d ($J = 2.0$ Hz, H-6), 6.42d ($J = 2.0$ Hz, H-8), 6.89s (B-ring $\underline{H}$), 8.06s (H-2), 13.09s (5-$O\underline{H}$).

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