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5,7,3′,4′,5′-PENTAOXYGENATED AND 2′,6′-DIPRENYLATED ISOFLAVONES FROM *PISCIDIA ERYTHRINA*

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Key Word Index—*Piscidia erythrina*; Leguminosae; isoflavonoids; 2′,6′-diprenyl isoflavone; 5,7,3′,4′,5′-pentaoxygenated isoflavones; 6′-prenylpiscerythrone.

Abstract—The structure of '6′-prenylpiscerythrone', a diprenylated isoflavone from *Piscidia erythrina*, has been unambiguously revised using chemical and spectroscopic methods to 5,7,3′,4′-tetrahydroxy-5′-methoxy-2′,6′-di-(3,3-dimethylallyl)isoflavone (erythbigenin). A further investigation of the root bark components of *P. erythrina* has also revealed the presence of three isoflavones with 5,7,3′,4′,5′-pentaoxygenation. These compounds have been identified as 5,7,3′,5′-tetrahydroxy-4′-methoxyisoflavone (junipegenin A), 5,7,4′-trihydroxy-3′,5′-dimethoxyisoflavone (piscigenin) and 5,7,4′,5′-tetrahydroxy-3′-methoxy-2′-(3,3-dimethylallyl)isoflavone (erythgenin).

INTRODUCTION

We have obtained ^{1}H NMR data [1] to suggest that an incorrect structure has been assigned to '6-prenylpiscerythrone' [5,7,2′,4′-tetrahydroxy-5′-methoxy-3′,6′-di-(3,3-dimethylallyl)isoflavone, **3**], an isoflavonoid found in the root bark of *Piscidia erythrina* L. [= *P. piscipula* (L.) Sarg.: Leguminosae–Papilionoideae] [2, 3]. Our attention was drawn to the structure of '6′-prenylpiscerythrone' as the result of a ^{1}H NMR study undertaken to establish if the presence, or absence, of 2′-hydroxylation in an isoflavone could be reliably deduced from the OH-5 chemical shift value [1]. Significantly, in the ^{1}H NMR spectrum of '6′-prenylpiscerythrone' the OH-5 signal (δ13.05) appeared at a position similar to that of piscidone **4** (OMe-2′, OH-5 at δ13.01 [1]). This result is incompatible with data recorded for 2′-hydroxy isoflavones with 5,7-dihydroxylation of ring A where the OH-5 signal was evident between δ12.51 and 12.79 (cf. piscerythrone **5**; with OH-2′, and OH-5 at δ12.55 [1]). Secondly, the ^{1}H NMR spectrum of 6′-prenylpiscerythrone' closely resembled that of the 2′,6′-diprenyl isoflavone piscerythramine (**2**) recently obtained from *P. erythrina* [4]. This observation was important because, as discussed later, the sidechain methyls of '6′-prenylpiscerythrone' exhibited chemical shift values difficult to explain on the basis of the 3′,6′-diprenyl substitution proposed in ref. [2].

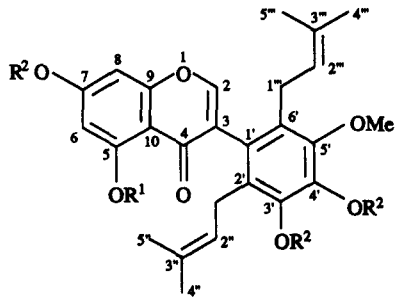
As the structure reported for '6′-prenylpiscerythrone' [2] was inconsistent with the ^{1}H NMR data, we decided to re-examine the compound using material extracted from *Piscidia* root bark recently collected in Mexico [5]. This study has now unambiguously revealed that '6′-prenylpiscerythrone' is 5,7,3′,4′-tetrahydroxy-5′-methoxy-2′,6′-di-(3,3-dimethylallyl)isoflavone (**1a**) and not 5,7,2′,4′-tetrahydroxy-5′-methoxy-3′,6′-di-(3,3-dimethylallyl)isoflavone (**3**) as reported by Delle Monache *et al.* [2]. The name erythbigenin is proposed for **1a** which is only the second known example of a sterically highly hindered 2′,6′-diprenylisoflavone, the first being piscerythramine (**2**) from *P. erythrina* [4].

A further investigation of the methanol soluble components of *P. erythrina* root bark also revealed the presence of three 5,7,3′,4′,5′-pentaoxygenated isoflavones (**6–8**) not previously found in this species. One of these compounds (**6**) is identical with the known *Juniperus* isoflavone junipegenin A [6].

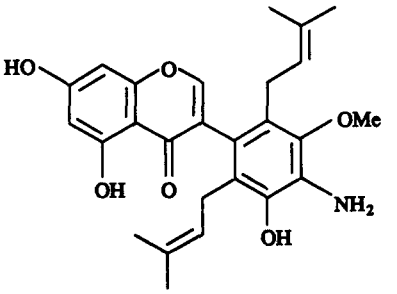
RESULTS AND DISCUSSION

During an investigation of the isoflavonoids in *Piscidia erythrina* root bark [5], we isolated substantial quantities of a compound recognizable as '6′-prenylpiscerythrone' from mp data, and a comparison of its spectroscopic properties (UV, mass spectrum, ^{1}H NMR) with those reported elsewhere [2, 3]. Our studies confirmed that the A- (H-6/H-8; 5,7-dihydroxy) and C- (H-2) rings of '6′-prenylpiscerythrone' had been correctly formulated in refs [2, 3]. Two prenyl (3,3-dimethylallyl) side chains and a methoxyl group were also identified, these being assigned (together with an OH group) to ring B which is thus fully substituted.

Upon methylation with dimethyl sulphate (see Experimental), '6′-prenylpiscerythrone' yielded both a trimethyl (**1b**) and a tetramethyl (**1c**) derivative (**1b**:**1c**, *ca* 2:1). In the presence of $AlCl_3$, the UV (MeOH) maximum (258 nm) of methyl ether **1b** shifted bathochromically by 10 nm confirming the presence of a 5-hydroxyl group [7]. In addition, high resolution ^{1}H and ^{13}C NMR data clearly revealed a symmetrical B-ring containing two equivalent prenyl sidechains, and a similarly equivalent pair of methoxyl substituents (see Experimental). Thus, the methylation experiment allows ring B of '6′-prenylpiscerythrone' to be represented by one of four possible part structures (**a–d**). However, as '6′-prenylpiscerythrone' has an asymmetric B-ring (see ^{1}H NMR data



1a $R^1 = R^2 = H$
1b $R^1 = H, R^2 = Me$
1c $R^1 = R^2 = Me$



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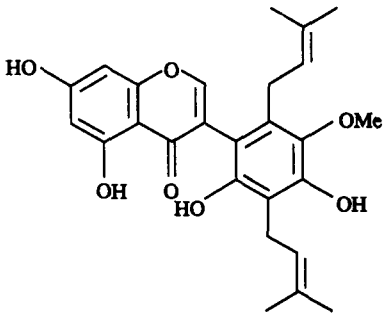

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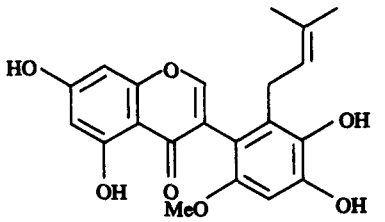

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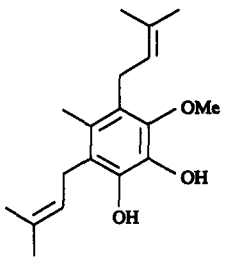
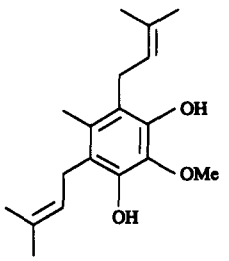


a



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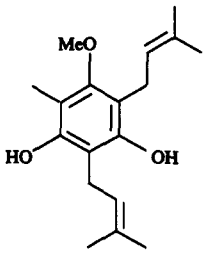
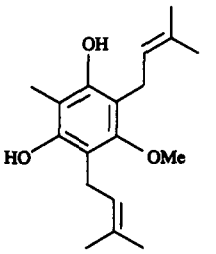


c



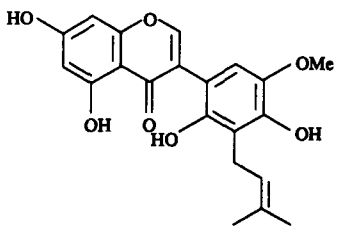
d

for the sidechains of **1a** in Table 1), the symmetrical part structures **b** and **d** can be completely discounted, leaving either **a** or **c** as the only possible alternatives.

Part structure **c** for ring B is implausible for the following reasons. Firstly, **c** contains a 2'-hydroxyl group which would be expected to exert a significant shielding effect on the OH-5 [1]. In the ^{1}H NMR spectrum of a 5,7-dihydroxy isoflavone possessing a **c**-type B-ring, the OH-5 signal should therefore appear at a position similar to that of OH-5 in piscerythrone (**5**, δ12.55 [1]) and not, as actually observed, at much lower field (δ13.04). Secondly, the methyl signals of prenyl sidechains located at C-3'/C-5' on ring B of an isoflavone (as shown in **c**) typically occur in the range δ1.60–1.80 (cf. the signals at δ1.65 and 1.78 for the sidechain methyls of piscerythrone **5** and licoisoflavone A) [2, 8]. In contrast, the methyl groups of prenyl sidechains located at C-2' (or C-6') appear at higher field (cf. those of piscidone **4** [5] and kwakhurin [9] at δ1.43/1.51 and δ1.39/1.49, respectively), exactly as seen in the ^{1}H NMR spectrum of '6'-prenylpiscerythrone' (ref. [2] and Table 1).

In order to obtain further evidence for the validity of the B-ring part structure **a**, '6'-prenylpiscerythrone' was cyclized using 99% HCO_2H as previously described [9]. A parallel reaction was also carried out on piscerythramine **2** [4'-amino-5,7,3'-trihydroxy-5'-methoxy-2',6'-di-(3,3-dimethylallyl)isoflavone] [4]. As expected, treatment of piscerythramine (**2**) with formic acid afforded three products (**9**–**11**), all containing a chromane ring formed by cyclization (6'→5' [O]) of the 6'-prenyl substituent. In compound **9** (M_r 451) the prenyl group at C-2' was unchanged, whereas in **10** (M_r 497) and **11** (M_r 469) this had been modified to give a 3-formyloxy-3-methylbutyl and a 3-hydroxy-3-methylbutyl sidechains, respectively.

When '6'-prenylpiscerythrone' was treated with formic acid in a similar manner to piscerythramine, the reaction mixture again yielded three products (**12**–**14**). The ^{1}H NMR characteristics of **12**–**14**, particularly with re-



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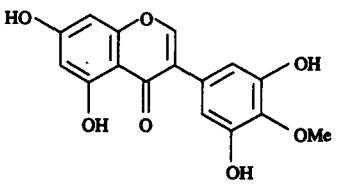

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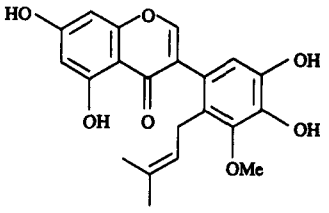

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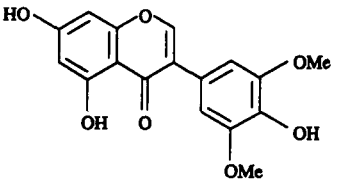

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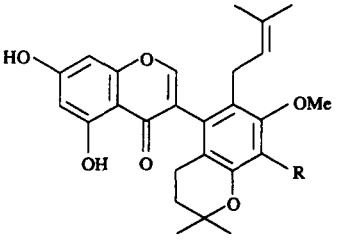
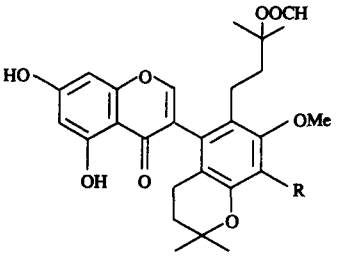
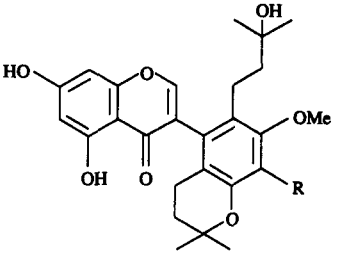


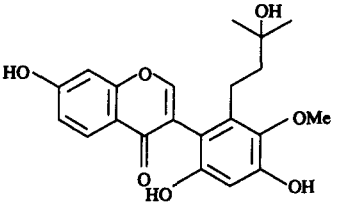
9 R = NH_2
12 R = OH



10 R = NH_2
13 R = OH



11 R = NH_2
14 R = OH



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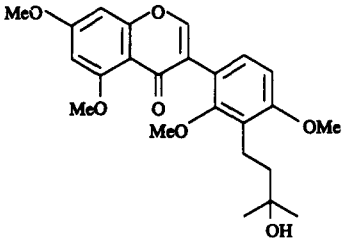

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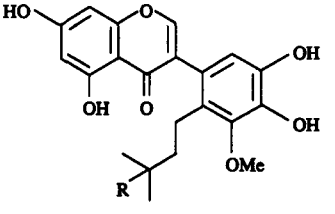

17 R = OH
18 R = OOCH

Table 1. ^{1}H NMR spectral data for compounds **1a** and **2** and their cyclization products (**9–14**) (acetone-d_6, 500 MHz, TMS)

H	**1a**	**2**	**12**	**9***	**13**	**10***	**14**	**11***
2	7.76 *s*	7.76*s*	7.88 *s*	7.86 *d-like* (*ca* 2)	7.98 *s*	7.96 *d-like* (1.5)	7.97 *s*	7.95 *d-like* (1.5)
OH-5	13.04 *s*	13.04 *s*	12.98 *s*	13.05 *s*	12.93 *s*	12.99 *s*	12.97 *s*	13.03 *s*
6	6.29 *d* (2.2)	6.29 *d* (2.2)	6.29 *d* (2.2)	6.28 *t-like* (*ca* 2)	6.30 *d* (2.1)	6.29 *t-like* (*ca* 2)	6.29 *d* (2.2)	6.28 *d-like* (2.0)
8	6.43 *d* (2.2)	6.43 *d* (2.2)	6.44 *d* (2.2)	6.43 *t-like* (*ca* 2)	6.45 *d* (2.1)	6.45 *t-like* (*ca* 2)	6.43 *d* (2.2)	6.43 *t-like* (2.0)
OMe-5′	3.73 *s*	3.78 *s*	3.81 *s*	3.70 *s*	3.87 *s*	3.75 *s*	3.86 *s*	3.75 *s*
1″a	3.29 *dd* (*ca* 15, 6)	3.19 *dd-like* (15, 7)	2.53 *dt* (16.9, 6.8)	2.50 *dt* (16.9, 6.8)	2.60–2.53 (2H) *m*	2.54 *dt* (*ca* 17, 7)	2.55 *dt* (16.9, 6.8)	2.52 *dt* (*ca* 17, 7)
1‴a	3.23 *dd* (*ca* 15, 7)	3.10 *dd-like* (15, 7)	3.23 *dd* (14.8, 7.8)	3.23 *dd-like* (*ca* 11, 7)		2.59–2.53 *t-like m*	2.59 *dt* (12.6, 4.7)	2.57 *dt* (*ca* 13, 5)
1″b	3.05 (2H)	2.96 (2H) *m*	2.42 *dt* (16.9, 6.8)	2.40 *dt* (17.0, 6.8)	2.43-2.34 (2H) *m*	2.37 *dt* (*ca* 17, 7)	2.40 *dt* (16.9, 6.8)	2.38 *dt* (*ca* 17, 7)
1‴b	*br dd* (*ca* 14, 7)		3.11 *dd* (14.8, 6.5)	3.11 *dd-like* (*ca* 11, 6)		2.41-2.36 *t-like m*	2.41 *dt* (12.6, 4.7)	2.42 (*ca* 13, 5)
H/H$_2$-2″	5.02 (1H) *br t-like* (*ca* 7)	4.95 (1H) *br t* (*ca* 7)	1.74 (2H) *t* (6.8)	1.72 (2H) *t* (6.8)	1.74 (2H) *br t* (*ca* 6)	1.73 (2H) *t* (*ca* 7)	1.74 (2H) *t* (6.8)	1.72 (2H) *t* (6.8)
H/H$_2$-2‴	4.95 (1H) *br t-like* (*ca* 7)	4.96 (1H) *br t* (*ca* 7)	4.95 (1H) *t-like* (6.8)	4.93 (1H) *br t-like* (*ca* 7)	1.90–1.84 (2H) *m*	1.87 (2H) *dt* (*ca* 12, 5)	1.60 (1H) *dt* (12.9, 4.6) 1.50 (1H) *dt* (12.9, 4.8)	1.59 (1H) *dt* (*ca* 11, 5) 1.51 (1H) *dt* (*ca* 11, 5)
H$_3$-4″	1.52 *s*	1.51 *s*	1.308 *s*	1.31 *s*	1.31 *s*	1.31 *s*	1.31 *s*	1.31 *s*
H$_3$-4‴	1.48 *s*	1.49 *s*	1.49 *s*	1.45 *s*	1.38 *s*	1.37 *s*	1.05 *s*	1.04 *s*
H$_3$-5″	1.36 *s*	1.37 *s*	1.306 *s*	1.31 *s*	1.30 *s*	1.30 *s*	1.30 *s*	1.30 *s*
H$_3$-5‴	1.35 *s*	1.35 *s*	1.38 *s*	1.35 *s*	1.35 *s*	1.34 *s*	1.04 *s*	1.03 *s*
Formyl-H	—	—	—	—	7.86 *s*	7.86 *s*	—	—

Coupling constants are given in Hz in parentheses.

*The reason for the unusual splitting of the H-2, H-6 and H-8 signals is unclear.

spect to their aliphatic (side structure) protons, very closely resembled those of compounds **9–11** resulting from acid-catalysed cyclization of piscerythramine (**2**) (Table 1). No evidence was obtained to suggest that acid treatment of '6'-prenylpiscerythrone' gave products in which a prenyl group had been cyclized in different directions (3'→ 2' [O] or 3'→4' [O]; cf. the response of licoisoflavone A [8]), or with two chroman rings (3'→2' [O]; 5'→4' [O]), as might be expected for a compound with a B-ring substituted as in part structure **c**.

Furthermore, in the ^{1}H NMR spectrum of **14** (Table 1), the methyl groups of the hydrated (3-hydroxy-3-methylbutyl) side chain appeared as 3H singlets at δ1.04 and 1.05. These chemical shift values are similar to those given by the 2' or 6'-sidechain methyls of kwakhurin hydrate (**15**; 1.01 *s*, 6H) [9] and cyclopiscerythramine hydrate (**11**; 1.03 *s* and 1.04 *s*, both 3H) (Table 1). In contrast, the methyl signals of a hydrated side chain located at C-3' (or C-5') are evident at lower field (e.g. 1.25, 6H for tetramethyl-licoisoflavone A hydrate, **16**) [9] close to, or identical with, those of the hydrated 6-sidechain (ring A) in luteone hydrate (1.26 *s*, 6H) [10, 11] and wighteone hydrate (1.26 *s*, 6H) [11], and the 8-sidechain in lupiwighteone hydrate (1.27 *s*, 6H) [11]. Further evidence for the 2',6'-diprenyl nature of '6'-prenylpiscerythrone' was provided by phase sensitive NOESY which afforded clear NOEs between 2-H (δ7.76) and the olefinic side chain protons (H-2'' and H-2''', δ5.02 and 4.95, respectively).

All the above ^{1}H NMR and chemical data effectively exclude the possibility that the B-ring of '6'-prenylpiscerythrone' is represented either by **c**, or the part structure (2'/4'-OH, 5'-OMe, 3'/6'-prenyl) suggested by Delle Monache *et al.* [2]. Because **a** is the only plausible B-ring alternative, the structure of '6'-prenylpiscerythrone' must be revised to 5,7,3',4'-tetrahydroxy-5'-methoxy-2',6'-di-(3,3-dimethylallyl)isoflavone (**1a**). We have named this compound erythbigenin because the correct substitution pattern is inconsistent with the name ('6'-prenylpiscerythrone') originally proposed [2].

In addition to erythbigenin, our studies also revealed three isoflavones with 5,7,3',4',5'-pentaoxygenation not previously found in *P. erythrina*. All co-occurred with piscidone (**4**) in fraction (Fr.) 16 obtained as reported earlier [5] by silica gel column chromatography of a methanolic extract of *P. erythrina* root bark. Each isoflavone was isolated from Fr. 16 by preparative silica gel TLC as described in the Experimental. The isoflavone nature of these *Piscidia* compounds was evident from their UV spectra in methanol (λ_{max} 260–265nm), and from the ^{1}HNMR singlet at δ7.9–8.3 (isoflavone H-2 [12]).

The most polar of the new *Piscidia* isoflavones (compound PE-16.1; R_f 0.14) afforded a base peak at *m/z* 316 ($[M]^+$ $C_{16}H_{12}O_7$) and a RDA fragment (*m/z* 153) indicative of a dihydroxylated A-ring [13]. Bathochromic shifts of the UV (MeOH) at 264nm upon addition of $AlCl_3$, or NaOAc, established that ring A is hydroxylated at C-5 and C-7, respectively [7]. Thus, compound PE-16.1 must possess a dihydroxymonomethoxy substituted B-ring in order to account for the ion at *m/z* 316 and the RDA (B-ring derived) fragment at *m/z* 164. The ^{1}HNMR spectrum of PE-16.1 revealed only two aromatic protons attributable to ring B. However, these resonated as a 2H singlet (δ6.80) which indicated that ring B is symmetrical with either a 2',6'-dihydroxy-4'-methoxy, or a 3',5'-dihydroxy-4'-methoxy, oxygenation pattern. The former possibility was discounted from the OH-5 NMR signal at δ13.08. As mentioned previously [1], the OH-5 chemical shift values of 2'-hydroxy isoflavones with 5,7-dihydroxylation on ring A vary from δ12.51 to 12.79 (seven compounds examined) whereas the OH-5 of corresponding 2'-deoxy (or 2'-*O*-derivatized) isoflavones occurs between δ12.99 and 13.09 (10 compounds examined). Compound PE-16.1 is therefore identical with 5,7,3',5'-tetrahydroxy-4'-methoxyisoflavone (junipegenin A, **6**) previously isolated from *Juniperus macropoda* (Cupressaceae) [6].

The second isoflavone from Fr. 16 (compound PE-16.2; R_f 0.17) had a M_r of 384 ($C_{21}H_{20}O_7$). 5,7-Dihydroxylation of ring A was established as described for junipegenin A, whilst a proton, a prenyl side chain, a methoxyl substituent and two hydroxyl groups were assigned to ring B from mass spectrometry (RDA fragments at *m/z* 232 and 177) and ^{1}H NMR data. The absence of a free hydroxyl group at C-2' was indicated by the ^{1}H NMR signal for OH-5 (δ12.98 [1]). As with the C-2' (or C-6') prenyl group in erythbigenin (**1a**), piscidone (**4**) [2, 5] and kwakhurin [9], the sidechain methyls of PE-16.2 occurred at higher field (δ1.43 and 1.50) than those of prenyl groups located at C-6, C-8 or C-3' (5') [8, 10, 11].

When heated with 88% HCO_2H, compound PE-16.2 gave both hydrate (**17**) and formyloxy (**18**) derivatives in yields of 56 and 41%, respectively (see Experimental for mass spectral data). This result indicated that neither of the B-ring hydroxyl groups was located *ortho* to the prenyl sidechain. As there was a clear NOE between 2-H and the single B-ring proton (6'-H), the structure of compound PE-16.2 must be 5,7,4',5'-tetrahydroxy-3'-methoxy-2'-(3,3-dimethylallyl)isoflavone (**7**) for which the name erythgenin is proposed. In **7** the H-6' NMR signal was observed (δ6.52) at higher than normal field (cf. H-6' of piscerythrone **5** at δ6.72 [2]) probably due to the steric effect of the 2'-prenyl group causing interference with coplanarity between the A/C- and B-rings.

The final isoflavone, PE-16.5 ($[M]^+$ *m/z* 330; $C_{17}H_{14}O_7$; R_f 0.44) resembled **6** and **7** in that ring A is hydroxylated at C-5 and C-7. Monohydroxy-dimethoxy substitution of ring B was evident from the RDA fragment at *m/z* 178 (cf. the ion at *m/z* 164 and 177 in the mass spectra of **6** and **7**, respectively) whilst the ^{1}H NMR spectrum established that the B-ring is symmetrical (two equivalent protons at δ6.94, and two equivalent methoxyl groups at δ3.87). As the OH-5 signal (δ13.07) indicated that C-2' is not hydroxylated [1], the equivalent B-ring protons must be located at C-2'/C-6' giving a 4'-hydroxy-3',5'-dimethoxy partial structure. Compound PE-16.5 is thus 5,7,4'-trihydroxy-3',5'-dimethoxyisoflavone (**8**). We suggest that **8** should be named piscigenin.

Compounds **6–8** are closely related to the 2',6'-diprenyl isoflavone erythbigenin (**1a**) in that all are 5,7,3',4',5'-penta-oxygenated. 6'-Prenylation of erythgenin (**7**) would in fact directly yield **1a**. To our knowledge, isoflavones with 5,7,3',4',5'-pentaoxygenation have not previously been recognized in the Leguminosae, although this oxygenation pattern occurs amongst isoflavones from the Iridaceae [14] and Cupressaceae [10, 15].

EXPERIMENTAL

Mps are uncorr. Instrumental analyses were carried out as described in refs [4, 5].

Plant material, extraction and isolation of isoflavonoids. Details of plant material, extraction procedure, initial CC and isolation

of isoflavone **1a** (erythbigenin ='6'-prenylpiscerythrone') are reported in ref. [5]. Erythbigenin was purified by crystallization from Me_2CO–EtOAc. Column Fr. 16 [5] was concd to give an oil (365 mg) from which crude piscidone (**4**, 9.5 mg) pptd. Upon multiple development (×3) silica gel prep. TLC in $CHCl_3$–MeOH (CM, 20:1) the above oil afforded 8 bands (PE-16.1–PE-16.8). Attention was concd on PE-16.1, PE-16.2 and PE-16.5 which fluoresced dark purple under long wavelength (365 nm) UV light. The lowest running material (band PE-16.1) was eluted (EtOAc) and purified by silica gel prep. TLC in CM (50:3, R_f 0.14) to yield, after crystallization from EtOAc, compound PE-16.1 (6.2 mg) as plates. After elution, band PE-16.2 was further chromatographed in CM (50:3, R_f 0.17) to give 5.9 mg of compound PE-16.2 as fine yellow rods from EtOAc. Finally compound PE-16.5 (7.1 mg R_f 0.44 in CM, 50:3) was isolated from band PE-16.5 by elution, prep. PTLC in $CHCl_3$–Me_2CO–conc NH_4OH (CAAm, 140:120:1, R_f 0.13) and crystallization from Me_2CO as fine rods.

Erythbigenin (**1a**). Amorphous powder, mp 214–217° (lit., 219–221° [2]; 214–216° [3]). UV_{365} nm fluorescence: dark purple. Gibbs test: (+), dark blue. UV λ_{max}^{MeOH} nm: 256sh, 258, 289(br); +NaOMe, 268, 325; +$AlCl_3$, 266, 310(br), 365(br); +NaOAc, 260, 288sh, 323sh and H_3BO_3 regenerated the MeOH spectrum. EIMS *m/z* (rel. int.): 453 $[M+1]^+$ (11), 452 $[M]^+$ (40), 396 (18), 384 (14), 383 (45), 381 (10), 257 (17), 244 (24), 229 (31), 153 (100), 69 (12). ^1H NMR, see Table 1. ^{13}C NMR δ (Me_2CO-d_6, 125 MHz): 182.5 (C-4), 165.0 (C-7), 163.6 (C-5), 159.3 (C-9), 155.8 (CH-2), 145.3 and 143.1 (C-3′ and C-5′), 138.6 (C-4′), 130.6 and 130.5 (C-3″ and C-3‴), 126.5 (C-3), 125.4 and 124.6 (C-2″ and C-2‴), 125.2 (C-1′), 122.8 and 122.7 (C-2′ and C-6′), 106.2 (C-10), 99.7 (CH-6), 94.3 (CH-8), 61.3 (OMe-5′), 27.1 and 27.0 (CH_2-1″ and CH_2-1‴), 25.7 and 25.6 (Me-4″ and Me-4‴), 17.5 and 17.4 (Me-5″ and Me-5‴).

Junipegenin A (PE-16.1, **6**). Plates, mp 260–262°. UV_{365} nm fluorescence: dark purple. Gibbs test: brown (rapid)→dark brown blue. UV λ_{max}^{MeOH} nm: 206, 220sh, 264, 295sh, 330sh; +NaOMe, 207, 271, 337, 365sh (br); +$AlCl_3$, 205sh, 226sh, 270, 300sh, 363; +NaOAc, 265sh, 275, 320sh and H_3BO_3 regenerated the MeOH spectrum. EIMS *m/z* (rel. int.): 317 $[M+1]^+$ (19), 316 $[M]^+$ (100), 315 (7), 301 (8), 287 (7), 245 (10), 243 (11), 242 (6), 217 (10), 164 (10), 153 (41), 149 (10), 146 (6), 128 (8), 121 (9), 69 (10). ^1H NMR δ (Me_2CO-d_6, 500 MHz): 13.08 (1H, *s*, OH-5), 8.20 (1H, *s*, H-2), 6.80 (2H, *s*, H-2′ and H-6′), 6.42 (1H, *d*, *J* = 2.0, H-8), 6.29 (1H, *d*, *J* = 2.0, H-6), 3.86 (3H, *s*, OMe-4′).

Erythgenin (PE-16.2, **7**). Pale yellow fine rods, mp 218–220°. UV_{365} nm fluorescence: dark purple. Gibbs test: yellow→dark blue. UV λ_{max}^{MeOH} nm: 208, 255sh, 260, 290sh (br), 325sh; +NaOMe, 207, 268, 326; +$AlCl_3$, 210, 266, 290–310 (br), 363; +NaOAc, 252sh, 267, 290sh, 330 and H_3BO_3 regenerated the MeOH spectrum. EIMS *m/z* (rel. int.): 385 $[M+1]^+$ (10), 384 $[M]^+$ (45), 369 (6), 341 (5), 328 (8), 316 (15), 313 (6), 301 (5), 232 (9), 177 (8), 154 (9), 153 (100), 69 (8). ^1H NMR δ (Me_2CO-d_6, 500 MHz): 12.98 (1H, *s*, OH-5), 7.92 (1H, *s*, H-2), 6.52 (1H, *s*, H-6′), 6.43 (1H, *d*, *J* = 2.0, H-8), 6.29 (1H, *d*, *J* = 2.0, H-6), 4.98 (1H, *br t*, *J* = 6.9, H-2″), 3.79 (3H, *s*, OMe-3′), 3.21 (2H, *br d*, *J* = *ca* 7, H_2-1″), 1.50 (3H, *d*, *J* = 1.0, H_3-5″), 1.43 (3H, *s*, H_3-4″). A clear NOE was observed on C-2-H or C-6′-H after irradiation at δ6.52 (H-6′) or δ7.92 (H-2).

Piscigenin (PE-16.5, **8**). Fine rods, mp 243–244°. UV_{365} nm fluorescence: dark purple. Gibbs test: brown→dark brown purple. UV λ_{max}^{MeOH} nm: 207, 218sh, 265, 295sh, 330sh (br); +NaOMe, 228sh, 270sh, 280 (br), 320sh (br); +$AlCl_3$, 228sh, 274, 305sh, 365; +NaOAc, 273, 327 (br) and H_3BO_3 regenerated the MeOH spectrum. EIMS *m/z* (rel. int.): 331 $[M+1]^+$ (17), 330 $[M]^+$ (100), 315 (8), 287 (15), 216 (9), 178 (6), 163 (5), 153 (20), 135 (10), 108 (5), 69 (6). ^1H NMR δ (Me_2CO-d_6, 500 MHz): 13.07 (1H, *s*, OH-5), 8.25 (1H, *s*, H-2), 6.94 (2H, *s*, H-2′ and H-6′), 6.43 (1H, *t*-like, *J* = 2.0, H-8), 6.30 (1H, *t*-like, *J* = 2.0, 6-H), 3.87 (6H, *s*, OMe-3′ and OMe-5′).

Methylation of erythbigenin (**1a**). A mixt. of **1a** (80 mg), K_2CO_3 (500 mg), Me_2SO_4 (100 μl) and Me_2CO (20 ml) was refluxed for 90 min and then worked up in the usual manner [8]. The resulting EtOAc ext was chromatographed (silica gel prep. TLC) in *n*-hexane–EtOAc (HE, 3:1) to yield 56 mg of the triMe ether (**1b**; R_f 0.47; FDMS, $[M]^+$ 494) and 28 mg of the tetraMe ether (**1c**; R_f 0.07; FDMS, $[M]^+$ 508).

Erythbigenin trimethyl ether (**1b**). Powder, mp 134–136°. UV_{365} nm fluorescence: dark purple. Gibbs test: (+), purple. UV λ_{max}^{MeOH} nm: 255sh, 258, 294 (br); +NaOMe, 261, 347 (br); +$AlCl_3$, 268, 312 (br), 366 (br); +NaOAc unchanged. EIMS *m/z* (rel. int.): 495 $[M+1]^+$ (8), 494 $[M]^+$ (27), 439 (11), 438 (16), 426 (19), 425 (72), 369 (8), 328 (20), 285 (30), 273 (9), 272 (26), 259 (11), 258 (9), 257 (41), 168 (10), 167 (100). ^1H NMR δ (Me_2CO-d_6, 500 MHz): 12.89 (1H, *s*, OH-5), 7.88 (1H, *s*, H-2), 6.58 (1H, *d*, *J* = 2.1, H-8), 6.38 (1H, *d*, *J* = 2.1, H-6), 4.95 (2H, *t*-like, *J* = 6.9, H-2″ and H-2‴), 3.93 and 3.91 (both 3H, two *s*, OMe-7 and OMe-4′), 3.74 (6H, *s*, OMe-3′ and OMe-5′), 3.26 (2H, *dd*, *J* = 14.7, 6.8, H_a-1″ and H_a-1‴), 3.06 (2H, *dd*, *J* = 14.7, 6.9, H_b-1″ and H_b-1‴), 1.51 (6H, *s*, H_3-4″ and H_3-4‴), 1.37 (6H, *s*, H_3-5″ and H_3-5‴). ^{13}C NMR δ (Me_2CO-d_6, 125 MHz): 182.2 (C-4), 166.7 (C-7), 163.4 (C-5), 159.0 (C-9), 155.9 (CH-2), 151.4 (C-3′ and C-5′), 147.4 (C-4′), 131.7 (C-2′ and C-6′), 131.0 (C-3″ and C-3‴), 127.1 (C-3), 124.6 (CH-2″ and CH-2‴), 122.5 (C-1′), 106.7 (C-10), 98.7 (CH-6), 92.9 (CH-8), 61.1 (OMe-3′ and OMe-5′), 60.7 (OMe-4′), 56.4 (OMe-7), 27.3 (CH_2-1″ and CH_2-1‴), 25.6 (Me-4″ and Me-4‴), 17.4 (Me-5″ and Me-5‴).

Erythbigenin tetramethyl ether (**1c**). UV_{365} nm fluorescence: blue-white. Gibbs test: (−). FDMS *m/z* 508 $[M]^+$ (100%). UV λ_{max}^{MeOH} nm: 246, 255sh, 284; +NaOMe, unchanged; +$AlCl_3$, unchanged. EIMS *m/z* (rel. int.): 508 $[M]^+$ (12), 439 (14), 328 (21), 285 (12), 182 (11), 181 (100).

Acid-catalysed cyclization of erythbigenin (**1a**). Crystallized **1a** (22 mg) was warmed (80°) for 3 hr in 2 ml of 99% HCO_2H, and the mixt. then worked-up as previously described [9]. Silica gel prep. TLC of the reaction products in CAAm (35:30:1) yielded three derivatives; **12**, R_f 0.25, 5.9 mg (27% yield), **13**, R_f 0.18, 7.1 mg (29% yield) and **14**, R_f 0.13, 9.0 mg (39%). For ^1H NMR see Table 1. Gibbs test: all (+), slow response, blue colour.

Compound **12**. Amorphous powder, mp 218–224°. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} nm: 254sh, 260, 289 (br); +NaOMe, 268, 329 (br); +$AlCl_3$, 267, 313 (br), 368 (br); +NaOAc, 255sh, 261, 270sh, 302 (br), 321 (br) and H_3BO_3 regenerated the MeOH spectrum. FDMS *m/z* (rel. int.): 453 $[M+1]^+$ (35), 452 $[M]^+$ (100). EIMS *m/z* (rel. int.): 453 $[M+1]^+$ (23), 452 $[M]^+$ (84), 397 $[M-55]^+$ (21), 396 (19), 384 (17), 383 (36), 381 (10), 342 (13), 300 (11), 244 (21), 229 (20), 153 (100), 69 (16).

Compound **13**. Amorphous powder, mp 168–175°. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} nm: 254sh, 261, 296 (br); +NaOMe, 268, 327 (br); +$AlCl_3$, 269, 312 (br), 364 (br); +NaOAc, 256sh, 261, 270sh, 302 (br), 326 (br) and H_3BO_3 regenerated the MeOH spectrum. FDMS *m/z* (rel. int.): 499 $[M+1]^+$ (32), 498 $[M]^+$ (100), 452 $[M-HCO_2H]^+$ (60). EIMS *m/z* (rel. int.): 453 $[M-45]^+$ (24), 452 $[M-HCO_2H]^+$ (84), 398 (15), 397 (62), 396 (22), 384 (34), 383 (36), 369 (10), 341 (33), 329 (17), 328 (24), 245 (13), 244 (19), 229 (19), 153 (100), 69 (15).

Compound **14**. Pale brown powder, mp 238–244°. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} (nm): 253sh, 261, 295 (br); +NaOMe, 268, 330 (br); +$AlCl_3$, 269, 312 (br), 371 (br); +NaOAc, 254sh, 261, 270sh, 302 (br), 325 (br) and H_3BO_3 regenerated the MeOH spectrum. FDMS *m/z* (rel. int.): 471 $[M+1]^+$ (35), 470 $[M]^+$ (100). EIMS *m/z* (rel. int.): 470 $[M]^+$ (25),

453 (12), 452 $[M-18]^+$ (41), 398 (17), 397 $[M-73]^+$ (75), 396 (43), 384 (16), 383 (25), 381 (15), 341 (32), 340 (14), 328 (10), 245 (12), 244 (21), 229 (21), 153 (100), 69 (13), 59 (12).

Acid-catalysed cyclization of piscerythramine (**2**). The cyclization of piscerythramine (**2**, 1.8 mg) was carried out as described for erythbigenin (**1a**) to yield three products (R_f in HE, 1:1) as follows: **9**, R_f 0.63, 0.5 mg (28% yield), **10**, R_f 0.50, 0.5 mg (25% yield) and **11**, R_f 0.13, 0.8 mg (43% yield). For ^{1}H NMR data see Table 1.

Compound **9**. Pale yellow gum. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} nm: 212, 254sh, 260, 292. FDMS *m/z* (rel. int.): 452 $[M+1]^+$ (30), 451 $[M]^+$ (100). EIMS *m/z* (rel. int.): 452 $[M+1]^+$ (36), 451 $[M]^+$ (100), 396 $[M-55]^+$ (12), 395 (14), 383 (10), 382 (23), 380 (12), 243 (26), 228 (17), 153 (46), 69 (11).

Compound **10**. Pale yellow gum. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} nm: 212, 254sh, 260, 292. FDMS *m/z* (rel. int.): 498 $[M+1]^+$ (42), 497 $[M]^+$ (100). EIMS *m/z* (rel. int.): 452 $[M-45]^+$ (39), 451 $[M-HCO_2H]^+$ (100), 397 (28), 396 (85), 395 (15), 382 (15), 380 (12), 341 (12), 340 (45), 328 (16), 325 (16), 244 (20), 243 (21), 228 (15), 153 (58), 69 (11), 46 (15), 45 (11).

Compound **11**. Pale yellow gum. UV_{365} nm fluorescence: dark purple. UV λ_{max}^{MeOH} nm: 212, 254sh, 261, 292. FDMS *m/z* (rel. int.): 470 $[M+1]^+$ (32), 469 $[M]^+$ (100). EIMS *m/z* (rel. int.): 470 $[M+1]^+$ (12), 469 $[M]^+$ (34), 452 (39), 451 $[M-18]^+$ (100), 397 (23), 396 $[M-73]^+$ (75), 395 (23), 383 (10), 382 (21), 380 (16), 352 (10), 341 (11), 340 (40), 339 (11), 328 (13), 326 (11), 325 (14), 244 (20), 243 (33), 228 (19), 154 (10), 153 (63), 69 (13).

Acid-catalysed cyclization of erythgenin (**7**). A mixt. of erythgenin (**7**, 2.4 mg), Me_2CO (2 drops) and 88% HCO_2H (0.3 ml) was warmed (62°) for 4 hr. The starting material (R_f 0.57 in $CHCl_3$–Me_2CO–EtOAc–MeOH [CAEM], 40:5:5:2) was gradually converted to two products (**17** and **18**) which were isolated by silica gel prep. TLC using CAEM.

Compound **17**. R_f 0.21 in CAEM, 40:5:5:2, 1.4 mg (56% yield). UV λ_{max}^{MeOH} nm: 207, 225sh, 254sh, 261, 285sh (br), 320sh (br); +NaOMe, 206, 225sh, 268, 325 (br); $AlCl_3$, 211, 260sh, 267, 305sh (br), 375 (br); +NaOAc, 268, 327 (br) and H_3BO_3 regenerated the MeOH spectrum. FDMS *m/z* (rel. int.): 403 $[M+1]^+$ (40), 402 $[M(=7+H_2O)]^+$ (100). EIMS *m/z* (rel. int.): 402 $[M]^+$ (14), 385 (5), 384 (20), 341 (5), 329 (22), 328 (30), 317 (6), 316 (10), 313 (11), 301 (13), 232 (8), 217 (6), 177 (7), 154 (10), 153 (100), 77 (6), 69 (15), 59 (12).

Compound **18**. R_f 0.50 in CAEM, 40:5:5:2, 1.1 mg (41% yield). UV λ_{max}^{MeOH} nm: 207, 225sh, 253sh, 260, 285sh (br), 320sh (br); +NaOMe, 205, 225sh, 268, 328 (br); +$AlCl_3$, 211, 258sh, 267, 305sh (br), 370 (br); +NaOAc, 269, 330 (br) and H_3BO_3 regenerated the MeOH spectrum. FDMS *m/z* (rel. int.): 431 $[M+1]^+$ (15), 430 $[M(=7+HCO_2H)]^+$ (19), 386 (18), 385 (44), 384 $[M-HCO_2H]^+$ (100). EIMS *m/z* (rel. int.): 385 (7), 384 $[M-HCO_2H]^+$ (30), 329 (14), 328 (13), 316 (9), 313 (5), 301 (10), 232 (8), 177 (7), 154 (9), 153 (100), 69 (11).

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