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ISOFLAVONES FROM PODS OF *LABURNUM ANAGYROIDES*

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Key Word Index—*Laburnum anagyroides*; Leguminosae; pods; isoflavonoids; isoflavones.

Abstract—The known isoflavones, genistein, 5-*O*-methylgenistein, wighteone, luteone and alpinumisoflavone, have been isolated from methanol extracts of *Laburnum anagyroides* pods. The pod extracts also yielded three new isoflavones; anagyroidisoflavone A (5,4′-dihydroxy-(3″,4″-dihydro-3″-hydroxy-4″-methoxy)-2″,2″-dimethylpyrano (5″,6″:6,7)isoflavone), anagyroidisoflavone B (5,4′-dihydroxy-(3″,4″-dihydro-3″,4″-epoxy)-2″,2″-dimethylpyrano (5″,6″:6,7)isoflavone) and laburnetin (5,7,4′-trihydroxy-6-(2″-hydroxy-3″-methyl-3″-butenyl)isoflavone). The structures of the new compounds were determined by spectral analyses.

INTRODUCTION

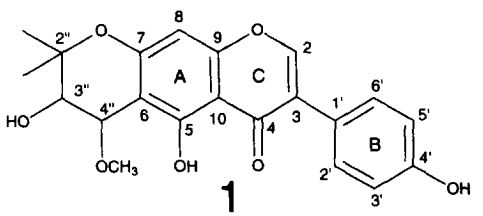
The common laburnum (*Laburnum anagyroides* Med. subfamily Papilionoideae) is widely grown as an ornamental in British gardens [1]. Although *L. anagyroides* is well known as a source of toxic alkaloids, its leaves and sapwood also contain the isoflavone, genistein (5,7,4′-trihydroxyisoflavone, **4**), as well as several related compounds [2–4].

In this paper, we describe the isolation and structural determination of three new isoflavones (anagyroidisoflavone A **1**, anagyroidisoflavone B **2** and laburnetin **3**), each having a genistein-type oxygenation pattern, from freshly collected *L. anagyroides* pods. Pod extracts also yielded genistein (**4**), in addition to alpinumisoflavone (**5**), wighteone (**6**), luteone (**7**) and 5-*O*-methylgenistein (**8**). Although alpinumisoflavone has previously been obtained from the heartwood of *L. alpinium* [4], it does not appear to have been found in *L. anagyroides*.

RESULTS AND DISCUSSION

As described in the Experimental, repeated column chromatography and preparative TLC of the methanol extracts of *L. anagyroides* pods gave eight isoflavones, three of which (**1**–**3**) have not previously been reported.

After lengthy purification, anagyroidisoflavone A (**1**) was obtained as a powder with $[M]^+$ (FD-mass spectrum) at *m/z* 384 (corresponding to $C_{21}H_{20}O_7$). The EI-masss spectrum gave fragments at *m/z* 352 $[M - OMe - H]^+$ and *m/z* 334 (base peak; $[M - OMe - H - H_2O]^+$), whilst the presence of 21 carbon atoms (one methoxyl, two methyls, eight methines and ten quaternary carbons) was established from the ^{13}C NMR and DEPT spectra. The identity of **1** as a 5-hydroxy-isoflavone was



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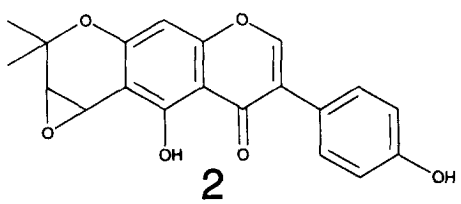

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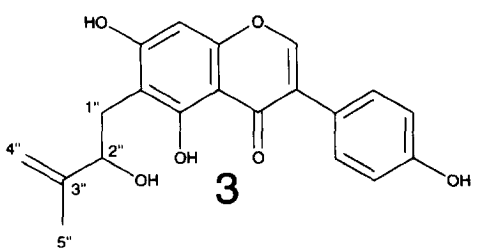

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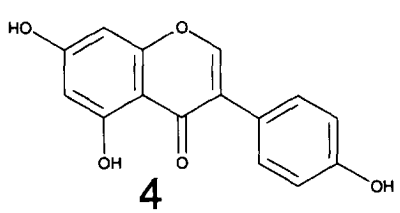

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deduced from the UV spectrum (λ_{max} 263 nm, shifting bathochromically with $AlCl_3$) and the ^{1}H NMR signals at δ8.19 (1H, *s*, H-2) and δ13.74 (C-5-OH) [5]. The UV maximum was unchanged by addition of NaOAc (C-7 OH absent) [5].

Apart from H-2 and OH-5, the ^{1}H NMR spectrum of **1** gave signals typical of a substituted 2,2-dimethyldihydropyrano unit, namely, two singlets at δ1.16 (3H) and 1.30 (3H) attributable to *gem*-dimethyl groups, an *A*, *B*-spin system at δ4.48 and δ5.16 (both 1H, *d*, J = 2.6 Hz), assigned to H-3″ and H-4″, and signals at δ3.48 (3H, OMe) and δ3.95 (1H, aliphatic OH). In addition to an aromatic proton signal at δ6.43 (1H, s), the ^{1}H NMR spectrum also revealed four aromatic protons (A_2B_2-system) at δ6.90 and 7.45 (both 2H, *d*, J = 8.5 Hz), which were assigned to a *p*-disubstituted benzene [5], as found (ring *B*) in genistein (**4**) and alpinumisoflavone (**5**). Since **1** gave a blue colour with Gibb's reagent [6], the methoxy-hydroxy-substituted side-structure must be linearly attached to ring *A*, with the position (C-8) *para* to the C-5–OH being unsubstituted (δ6.43, H-8) [5, 7]. HMBC data indicated the following correlations: *gem*-dimethyl protons (2 × Me-2″ at δ1.16 and 1.30) to C-2″ (δ70.87) and C-3″ (δ97.86), methoxy protons (δ3.48) to C-4″ (δ79.08) and H-4″ methine proton (δ5.16) to C-2″ (δ70.87) and C-3″ (δ97.86). These correlations suggest that the OMe and OH substituents on the side-structure are located at C-4″ and C-3″, respectively. Acetylation of **1** resulted in a shift of the H-3″ (δ4.48) signal to lower field (δ4.53 in the 4′-monoacetate and δ4.56 in the 4′,3″-diacetate), which allows this proton to be assigned to a carbon with a secondary alcohol group [8]. Compound **1** is therefore 5,4′-dihydroxy-(3″,4″-dihydro-3″-hydroxy-4″-methoxy)-2″,2″-dimethylpyrano(5″, 6″:6, 7)isoflavone (*anagyroidisoflavone A*). Acetone and fresh methanol extracts of *L. anagyroides* pods were concentrated *in vacuo* and the residue was chromatographed (silica gel TLC, two solvent systems) in order to detect **1**.

Anagyroidisoflavone B (**2**), isolated from *Laburnum* pods in only trace amounts, had an isoflavone-like UV (MeOH) spectrum with λ_{max} 267 nm shifting to 279 nm after addition of $AlCl_3$ (C-5–OH). No UV shift occurred with NaOAc (C-7 OH absent).

The ^{1}H NMR spectrum (Table 1) confirmed that rings B (aromatic) and C (heterocyclic) of **2** were identical to those of **1**. As **2** gave a blue Gibb's test colour, the ^{1}H NMR signal at δ6.32 (1H, *s*) was assigned to H-8. Thus, **2** differs from **1** only in the nature of its linear (A-ring, C-6/7) side attachment, which gave a ^{1}H NMR singlet at δ1.27 (6H, *gem*-dimethyl-2″) and two 1H doublets at δ3.94 and 4.37 (both J = 3.3 Hz, H-3″ and 4″, respectively). Since the HR-mass spectrum of **2** gave $[M]^+$ 352.0922 (corresponding to $C_{20}H_{16}O_6$), the above spectroscopic data are best explained if the sixth oxygen atom occurs in the side structure as an epoxide ring (C-3″/4″). This view is supported by the mass spectral fragment observed at *m/z* 334 $[M - 18]^+$ [9]. Compound **2** is, therefore, 5,4′-dihydroxy-(3″,4″-dihydro-3″,4″-epoxy)-2″,2″-dimethylpyrano(5″,6″:6,7)isoflavone (*anagyroidisoflavone B*).

The third *Laburnum* compound (laburnetin, **3**) was obtained as a powder with $[M]^+$ (HR-mass spectrum) at *m/z* 354.1110 ($C_{20}H_{18}O_6$). Like **1** and **2**, laburnetin was identified (UV and ^{1}H NMR) as a 5-hydroxy-isoflavone, with genistein-like-B- and C-rings. However, in contrast to **1** and **2**, the UV maximum (MeOH, λ_{max} 266 nm) of **3** shifted bathochromically by 7 nm after addition of NaOAc (C-7–OH). The presence of a 2-hydroxy-3-methyl-3-butenyl side-chain, as previously found in dolichins A and B, and in lupinisols A, B and C [10–12], was evident from the ^{1}H NMR signals at δ2.93 and 3.08 (both 1H, *dd*, 1″-Ha/1″-Hb), δ4.40 (1H, *dd*, 2″-H), δ4.75 and

Table 1. ^{1}H NMR data (δ values) for **1–3**

Proton	Anagyroidisoflavone A **1**	Anagyroidisoflavone B **2**	Laburnetin **3**
2	8.19 (1H, *s*)	8.16 (1H, *s*)	8.12 (1H, *s*)
8	6.43 (1H, *s*)	6.32 (1H, *s*)	6.42 (1H, *s*)
2′, 6′	7.45 (2H, *d*, J = 8.5)	7.45 (2H, *d*, J = 8.5)	7.45 (2H, *d*, J = 8.7)
3′, 5′	6.90 (2H, *d*, J = 8.5)	6.89 (2H, *d*, J = 8.5)	6.90 (2H, *d*, J = 8.7)
1″	—	—	2.93 (1H, *dd*, J = 8.0, 14.2) 3.08 (1H, *dd*, J = 3.5, 14.2)
2″	—	—	4.40 (1H, *dd*, J = 3.5, 8.0)
3″	4.48 (1H, *d*, J = 2.6)	4.37 (1H, *d*, J = 3.3)	
4″	5.16 (1H, *d*, J = 2.6)	3.94 (1H, *d*, J = 3.3)	4.75 (1H, *br s*) 4.95 (1H, *br s*)
5″	—	—	1.83 (3H, *s*)
5-OH	13.74 (1H, *s*)	13.64 (1H, *s*)	13.50 (1H, *s*)
4′-OH	8.53 (1H, *s*)	8.54 (1H, *s*)	8.50 (1H, *br s*)
2″-Me	1.16 (3H, *s*) 1.30 (3H, *s*)	1.27 (6H, *s*)	—
3″-OH	3.95 (1H, *s*)	—	—
4″-OMe	3.48 (3H, *s*)	—	—

Spectra determined at 500 MHz (acetone-d_6), J in Hz.

4.95 (both 1H, *br s*, 4″-H_2) and δ1.83 (3H, *s*, 5″-H) (Table 1). As **3** gave a blue colour with Gibb's reagent, the side-chain was placed at C-6 and the aromatic A-ring proton (δ6.42) at C-8. Compound **3** is thus 5,7,4′-trihydroxy-6-(2″-hydroxy-3″-methyl-3″-butenyl)isoflavone (*laburnetin*).

In addition to **1–3**, the pod extracts also afforded five other isoflavones. These were identified as genistein, alpinumisoflavone, wighteone, luteone and 5-*O*-methylgenistein from spectroscopic data (UV, mass spectra, ^{1}H NMR) and TLC comparison with authentic material [5, 13, 14]. Although alpinumisoflavone has been found in *L. alpinum* [13], this compound does not appear to have been obtained previously from *L. anagyroides*.

EXPERIMENTAL

General. Unless indicated otherwise, NMR spectra were measured in acetone-d_6 (TMS int. standard) at 125 MHz (^{13}C) or 500 MHz (^{1}H). EI and FD MS were obtained using a direct inlet. UV spectra were run in MeOH. Analytical and prep. TLC sepns were carried out on Merck pre-coated silica gel 60 plates using $CHCl_3$–MeOH (9:1) (CM), benzene–EtOAc (4:1) (BE), $CHCl_3$–Me_2CO–aq.NH_4OH (35:30:1) and *n*-pentane–Et_2O–HOAc (75:25:4).

Extraction and purification. Pods of *L. anagyroides* Med. were collected in August 1986 from verified trees growing at the Royal Botanic Garden, Kew. Fresh pods (972 g) were opened and seeds removed. Empty pods (502 g) were then immersed for 3 days, in twice their vol. of MeOH. After filtration, the green MeOH extract (9 l) was concd *in vacuo* (40°) and the residue (40 g) chromatographed on a Kieselgel 60 column (429 g) using $CHCl_3$–MeOH with increasing amounts of MeOH. Eluates (250 ml per fr.) were collected as follows: (frs 1–3, 1% MeOH in $CHCl_3$; frs 4–11, 2% MeOH in $CHCl_3$; frs 12–20, 3.5% MeOH in $CHCl_3$; frs 21–25, 8% MeOH in $CHCl_3$ and frs 26–29, 10% MeOH in $CHCl_3$.

Preliminary TLC examination (CM) of frs 4 and 5 indicated the presence of only one isoflavone (**5**, R_f 0.71). Frs 4 and 5 were combined and evapd to dryness. The residue (940 mg) was subjected to CC over Kieselgel 60 (15 g) prepd in $CHCl_3$ (15 ml per fr. frs 1′–15′). Prep. TLC of combined frs 5′ and 6′ (BE) gave a band of fluorescence-quenching material at R_f 0.51. This was eluted with Me_2CO and yielded alpinumisoflavone **5**.

Frs 6–10 were combined and concd to give a brown residue (330 mg). This was chromatographed (prep. TLC) in BE to yield fluorescence-quenching material at R_f 0.51 (**5**), 0.26 (**6**), 0.20 (**3**) and 0.08 (**1**). Each band was eluted and upon further prep. TLC in CM afforded alpinumisoflavone **5**, wighteone **6** (R_f 0.40), laburnetin **3** (R_f 0.37) and anagyroidisoflavone A **1** (R_f 0.41).

Frs 11–15 were also combined and evapd to dryness. The residue (410 mg) was dissolved in a minimum vol. of MeOH and the soln chromatographed (prep. TLC) in BE to yield 7 bands at R_fs 0.51 (**5**), 0.26 (**6**), 0.20 (**3**), 0.16 (**4**), 0.10 (unidentified) 0.12 (**2**) and 0.08 (**1**). Elution and further prep. TLC of each band in CM, gave alpinumisoflavone **5**, wighteone **6**, laburnetin **3**, genistein **4** (R_f 0.34), anagyroidisoflavone B **2** (R_f 0.39) and anagyroidisoflavone A **1**.

Frs 16 and 17 were combined and concd (180 mg) and then chromatographed (prep. TLC in BE) to give 3 major bands at R_fs 0.26, 0.16 and 0.11. After elution, components of these bands were rechromatographed (prep. TLC in CM) to afford wighteone **6**, genistein **4** and luteone **7** (R_f 0.32). Fr. 19 was subjected to repeated prep. TLC in CM (R_f 0.15) to yield pure 5-*O*-methylgenistein **8**.

Final isoflavone yields were anagyroidisoflavone A (**1**, 6.8 mg), anagyroidisoflavone B (**2**, 0.4 mg), laburnetin (**3**, 10.4 mg) genistein (**4**, 23.6 mg), alpinumisoflavone (**5**, 23.7 mg), wighteone (**6**, 55 mg), luteone (**7**, 1.3 mg) and 5-*O*-methylgenistein (**8**, 18 mg). Compounds **4–8** were identified by UV, MS, ^{1}H NMR and TLC (4 solvent systems) comparison with authentic material and lit. values.

Anagyroidisoflavone A (**1**). Powder. Gibb's reagent: (+)sky-blue. FD-MS *m/z* (rel. int.): 384 $[M]^+$ (100), 352 (26.1), 294 (10.9). EIMS: 352.0943 $[M-32]^+$ ($C_{20}H_{16}O_6$, calc. 352.0944) (2.1), 334 $[M-32-18]^+$ (100), 294 (6.9), 216 (2.2) 167 (1.4), 118 (1.0). UV λ_{max} nm: 210, 263; + $AlCl_3$ 215 (sh), 272; + NaOAc unchanged. ^{13}C NMR (125 MHz, acetone-d_6): δ24.9 and 26.6 (2″-Me_2), 57.0 (4″-OMe), 79.1 (C-4″), 89.7 (C-8), 97.9 (C-3″), 115.9 (C-3′, C-5′), 131.2 (C-2′, C-6′), 154.4 (C-2), 70.9 (C-2″) 106.8 (C-10), 111.0 (C-6), 122.8 (C-1′), 124.0 (C-3), 158.4 (C-4′), 160.2 (C-5, C-9), 168.4 (C-7), 182.0 (C-4). HMBC data in which the following correlations were observed: H-2 → C-3, C-4 and C-9; 5-OH → C-6 and C-10; H-8 → C-6, C-7 and C-10; H-2′,6′ → C-3 and C-4′; H-3′,5′ → C-1′; 4′-OH → C-3′ and C-5′; 2″-2 × Me → C-2″ and C-3″; H-3″ → C-4″ → C-4″; H-4″ → C-2″ and C-3″; 4″-OMe → C-4″. ^{1}H NMR, see Table 1.

Acetylation of **1**. Compound **1** (3 mg) was treated with Ac_2O–pyridine (1:1, 1 ml) for 24 hr at room temp. and worked-up in the usual manner. The two major products were purified by prep. TLC in CM. 4′-monoacetate. FD MS *m/z* (rel. int.): 426 $[M]^+$ (100) 394 (88), 384 $[M-42]^+$ (11). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.25 and 1.32 (each 3H, *s*, 2″-Me × 2), 2.32 (3H, *s*, 4′-OCOMe), 3.57 (3H, *s*, 4″-OMe), 4.53 (1H, *d*, J = 1.6 Hz, H-3″), 5.11 (1H, *d*, J = 1.6 Hz H-4″) 6.42 (1H, *s*, H-8), 7.18 (2H, *d*, J = 8.4 Hz, H-3′, H-5′), 7.56 (2H, *d*, J = 8.4 Hz, H-2′, H-6′), 7.89 (2H, *s*, H-2), 13.49 (1H, *s*, 5-OH).

4′,3″-Diacetate. FD MS *m/z* (rel. int.): 468 $[M]^+$ (100), 426 $[M-42]^+$ (87), 394 (39), 384 $[M-42-42]^+$ (19). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.26 and 1.28 (each 3H, *s*, 2″-Me × 2), 2.31 (3H, *s*, 4′-OCOMe), 2.44 (3H, *s*, 3″-OCOMe), 3.39 (3H, *s*, 4″-OMe), 4.56 (1H, *d*, J = 2.0 Hz, H-3″), 5.11 (1H, *d*, J = 2.0 Hz, H-4″), 6.79 (1H, *s*, H-8), 7.13 (2H, *d*, J = 8.6 Hz, H-3′, H-5′), 7.48 (2H, *d*, J = 8.6 Hz, H-2′ and H-6′), 7.81 (1H, *s*, H-2), 13.43 (1H, *s*, 5-OH).

Anagyroidisoflavone B (**2**). Powder, Gibb's reagent: (+) sky-blue. EIMS *m/z* (rel. int.): 352.0922 $[M]^+$ ($C_{20}H_{16}O_6$, calc. 352.0944) (5.2), 334 $[M-18]^+$ (100), 283 (2.9), 216 (16), 167 (12), 89 (4.7). UV λ_{max} nm: 267; + $AlCl_3$ 220 (sh), 279; + NaOAc unchanged. ^{1}H NMR, see Table 1.

Laburnetin (3) *Powder*. Gibb's reagent: (+) Prussian blue. EIMS *m/z* (rel. int.): 354.1110 $[M]^+$ ($C_{20}H_{18}O_6$, calc. 354.1104) (3.9), 336 $[M - 18]^+$ (10), 321 $[M - 18 - 15]^+$ (30), 284 (38), 283 $[M - 71]^+$ (100), 165 (6.1), 123 (6.2), 118 (5.2). UV λ_{max} nm: 212, 266; + $AlCl_3$ 215 (sh), 273; + NaOAc 273, 335. ^{13}C NMR (125 MHz, acetone-d_6): δ 18.3 (C-5″), 23.2 (C-1″), 110.4 (C-4″), 76.4 (C-2″), 94.9 (C-8), 115.9 (C-3′, C-5′), 131.2 (C-2′, C-6′), 154.2 (C-2), 110.2 (C-6, C-10), 122.9 (C-1′), 123.2 (C-3), 148.2 (C-3″), 154.2 (C-9), 157.3 (C-4′), 158.3 (C-5), 164.3 (C-7), 181.7 (C-4). ^1H NMR, see Table 1.

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