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ISOFLAVONES AND XANTHONES FROM *POLYGALA VIRGATA*

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Key Word Index—*Polygala virgata*; Polygalaceae; chemotaxonomy; isoflavones; xanthones.

Abstract—Three isoflavones and two xanthones have been isolated from the roots of *Polygala virgata*. The structures were established on the basis of spectral data of the isolates and of some derivatives. 5,7,4'-Trihydroxy-6,8-dimethoxyisoflavone and 5,7-dihydroxy-6,8,4'-trimethoxyisoflavone are new natural products, whereas 5,7-dihydroxy-8,4'-dimethoxyisoflavone, although known synthetically, is reported here for the first time as a naturally occurring compound. The isolated xanthones (1,7-dihydroxy-2,3,4-trimethoxyxanthone and 1,7-dihydroxy-3,4-dimethoxyxanthone) have been described previously in other species.

INTRODUCTION

The genus *Polygala* (Polygalaceae) comprises *ca* 500 species and is distributed over temperate, subtropical and tropical regions of all continents with the exception of Australia [1]. Previous phytochemical investigations of the genus have shown the presence of a broad spectrum of structurally and biogenetically diverse secondary metabolites such as saponins [2], lignans [3], coumarins [4], xanthones [5], hydroxycinnamic esters [6], flavonol glycosides [7] and chromonocoumarins (=6*H*,7*H*-[1]benzopyrano[4,3-*b*][1]benzopyran-6,7-diones) [8]. As a part of our ongoing search for biologically active natural products from *Polygala* species, we undertook an investigation of *P. virgata*. It is a herb or small shrub up to 1.5 m tall which is quite common in South Africa and on the high plateaux of Malawi, where it grows in woodland and on the edges of the forests. Previous studies on the seed oil composition of this species revealed the presence of acetylated triglycerides [9], but there has been no report on secondary metabolites so far. Preliminary TLC and HPLC analysis of the lipophilic root extract suggested the presence of flavonoids and xanthones. We report on the isolation and structure elucidation of isoflavones and xanthones from *P. virgata*.

RESULTS AND DISCUSSION

Fractionation of the dichloromethane extract on silica gel, followed by gel filtration on Sephadex LH-20 (MeOH), semi-prep. HPLC on RP-18 and CN columns afforded compounds **1**–**5**. The UV spectrum of **1** exhibited four absorption bands characteristic of a xanthone. A bathochromic shift observed upon addition of $AlCl_3$ indicated the presence of a chelated hydroxyl group at C-1. The lack of free hydroxyl groups at C-3 and C-6 was evidenced by the absence of a shift with NaOAc. The ^{1}H NMR spectrum (see Experimental), showed the presence of two methoxyl groups (δ3.93 and 3.89). A doublet at δ7.42 (J=9.1 Hz, H-5), a doublet of doublets at δ7.25 (J=9.1, 2.9 Hz, H-6) and a doublet at δ7.49 (J=2.9 Hz, H-8) indicated substitution at C-7. A singlet at 6.37 ppm was attributable to H-2 in a 1-hydroxy-3,4-dimethoxy substituted xanthone [10]. In the 1-hydroxy-2,3-dimethoxy-isomers, H-4 appear around δ6.50–6.56 [11–13]. In the ^{13}C NMR spectrum (see Experimental), the signal at δ60.8 was characteristic of an *ortho* disubstituted methoxyl group (MeO-4), whereas the signal at δ56.5 was attributable to an *ortho* mono- or non-substituted methoxyl group [14]. The position of the second methoxyl group was located with the aid of NOE difference experiments. Presaturation of H-2 gave enhancement of the signal at δ3.93, confirming the presence of a methoxyl group at C-3. Thus, **1** was identified as 1,7-dihydroxy-3,4-dimethoxyxanthone (veratrilogenin), previously isolated from *Veratrilla baillonii* (Gentianaceae) [15].

On the basis of UV, ^{1}H NMR, ^{13}C NMR and EI mass spectral data, the structure of **2** was established as 1,7-dihydroxy-2,3,4-trimethoxyxanthone. This compound was first reported from *Frasera speciosa* (Gentianaceae) [13].

The UV spectrum of **3** showed absorption maxima at 271 and 335 nm, respectively, suggesting an isoflavone nucleus rather than a flavone-type structure. The presence of hydroxyl groups at C-5 and C-7 was indicated by a bathochromic shift of band II with $AlCl_3$ and NaOAc, respectively. The ^{1}H NMR (DMSO-d_6) spectrum of **3** displayed signals of two aromatic methoxyl groups (δ3.8 and 3.79), an AA'BB' system (δ7.38, H-2' and H-6 and δ6.82, H-3' and H-5') of a *para* substituted aromatic ring, two hydroxyl groups (δ12.78 and 10.40, HO-5 and HO-7) and a singlet at δ8.40 (H-2). The isoflavone nucleus and the positions of the remaining substituents were confirmed with the aid of EI mass spectrometry, ^{13}C NMR and NOE difference measurements. Indeed, the large chemical shift difference observed for H-2 δ8.40 in **3** (DMSO-d_6), δ7.90 in **3a** ($CDCl_3$)) is typical of isoflavones. In addition to this, strong NOE's were observed between H-2 and H-2'/H-6'. In the ^{13}C NMR spectrum of **3**, signals attributable to C-2 (δ153.9) and C-3 (δ121.7) were

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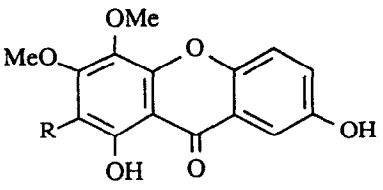
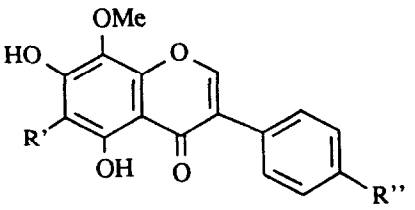


1 R = H

2 R = OMe



	R'	R''
3	OMe	OH
4	OMe	OMe
5	H	OMe

in good agreement with data reported for isoflavones. The presence of two *ortho* disubstituted methoxyl groups (δ61.0 and 60.0) indicated that they are attached to C-6 and C-8. The EI mass spectrum of **3** showed a $[M]^+$ at *m/z* 330. The fragment ions at *m/z* 118 $[B_1]^+$ and 169 $[A_1-43]^+$ supported the presence of a hydroxyl group in ring B and two methoxyl groups in the ring A. Thus, the structure of **3** was established as 5,7,4′-trihydroxy-6,8-dimethoxyisoflavone, a novel natural product.

The UV spectrum of **4** was similar to that of **3**. The shifts induced by $AlCl_3$ and NaOAc were characteristic of 5,7-dihydroxyisoflavones. In the ^{1}H NMR ($CDCl_3$) spectrum, a singlet at 7.93 ppm (H-2) was typical of isoflavones. A pair of *ortho* coupled doublets, each integrating for two protons, at δ7.45 (H-2′ and H-6′) and 6.98 (H-3′ and H-5′) was indicative of 4′ substitution. Three aromatic methoxyl groups appeared at δ4.03, 3.97 and 3.84. The EI mass spectrum of **4** showed a $[M]^+$ at *m/z* 344. The presence of two methoxyl groups in ring A and a third methoxyl group in ring B was evidenced by the fragment ions at *m/z* 169 $[A_1-43]^+$ and 132 $[B_1]^+$. In the ^{13}C NMR spectrum, signals at δ152.5 and 122.7 were attributable to C-2 and C-3, respectively. Signals at δ61.9 and 61.0, characteristic of *ortho* disubstituted methoxyl groups, were assigned to MeO-6 and MeO-8. The signal at δ55.4 was attributed to MeO-4′. Thus, **4** was characterized as 5,7-dihydroxy-6,8,4′-trimethoxyisoflavone, a new natural compound. The UV spectra of **5** recorded with the usual shift reagents were virtually identical to those of isoflavone **4**. The EI mass spectrum exhibited a $[M]^+$ at *m/z* 314. The presence of one methoxyl group in ring A was evidenced by the ions at *m/z* 167 $[A_1-15]^+$ and 139 $[A_1-43]^+$. The fragment ion at *m/z* 132 $[B_1]^+$, resulting from a retro-Diels–Alder cleavage of the parent ion, was typical of a methoxylated B-ring in isoflavones. In the ^{1}H NMR spectrum of **5**, one proton singlet at δ12.59 was attributable to the hydroxyl group at C-5. The H-2 signal at δ7.91 (in $CDCl_3$) exhibited a marked downfield shift of δ0.54 when the spectrum was measured in DMSO-d_6. An AA′BB′ system was indicative of a *para* substituted B-ring. The chemical shift of H-6 (6.32 ppm) was in good agreement with the data reported for analogous structures [16]. In the positional isomer (irisolidone), H-8 is slightly more deshielded (6.48 ppm) [17]. Unambiguous confirmation of the structure was obtained through NOE difference experiments. Presaturation of H-2 gave enhancement of H-2′/H-6′. Enhancement of H-6 was observed upon irradiation of the signal at δ12.59 (HO-5), indicating that the second methoxyl group was attached to C-8. Thus, **5** was identified as 5,7-dihydroxy-8,4′-dimethoxyisoflavone, a compound synthesized earlier as an isomer of irisolidone [17], but reported from nature for the first time.

There has been extremely few phytochemical studies on African *Polygala* species. Our investigations on lipophilic constituents of *Polygala virgata* afforded three isoflavonoids and two known xanthones, although the latter have not been reported from other *Polygala* species. Indeed, the oxygenation pattern of **1** (1,3,4,7) differs from those encountered so far in xanthones isolated from American and Indian *Polygala* species (1,2,3, 1,2,4, 1,3,4, 1,2,3,4, 1,2,3,7, 1,2,3,4,7, 1,3,6,7, 1,2,4,6,7 and 1,2,3,7,8) [5]. Isoflavonoids, a class of compounds typical of the Leguminosae and occasionally found in other families [18], are here reported for the first time from a species belonging to the Polygalaceae. The scant information about the flavonoid distribution within that family [19, 20] precludes at the moment the use of isoflavonoids as chemotaxonomic markers for the Polygalaceae and in particular of the genus *Polygala*.

EXPERIMENTAL

General. Mps are uncorr. TLC was carried out on silica gel pre-coated Al sheets (Merck) and on RP-18 HPTLC plates (Merck). The solvent systems employed were: $CHCl_3$–MeOH (19:1) (silica gel), and MeOH–H_2O (7:3) (RP-18). For open CC, silica gel (40–63 μm, Merck) was used. UV spectra were recorded with addition of usual shift reagents [21]. Semi-prep. HPLC was performed on a Lichroprep RP-18 column (7 μm, 16 × 250 mm, i.d., Knauer) at a flow rate of 10 ml min^{-1} and on a Lichrosorb CN column (7 μm, 4.6 × 250 mm, i.d., Knauer) at a flow rate of 2 ml min^{-1}. Purity of compounds was checked by HPLC on Lichrosorb RP-8, RP-18 and CN columns (7 μm, 4.6 × 250 mm, i.d., Knauer). ^{1}H and ^{13}C NMR spectra were measured at 200.6 MHz in DMSO-d_6 or $CDCl_3$. TMS was used as int. standard. NOE difference expts were performed according to ref. [22].

Plant material. P. virgata was collected in November 1989 on the Nyika Plateau, Malawi. The plant was identified by Mr E. A. Banda (National Herbarium of Malawi) and a voucher specimen is deposited at the National Herbarium, Zomba, Malawi.

Extraction and isolation. Dried roots (74 g) were successively extd at room temp. with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (1.4 g) was fractionated by CC on silica gel (4.5 × 60 cm) into 9 frs (1–9) using gradient elution ($CHCl_3$–MeOH, 97:3→4:1, followed by MeOH). Further sepn of fr 2 (155 mg) on Sephadex LH-20 (MeOH) yielded 4 frs (A–D). Fr C (31 mg) was pptd by the addition of MeOH. The ppts were filtered. The

residue consisted of pure **1** (4 mg). Further sepn of the filtrate of fr C by semi-prep. HPLC (RP-18, MeOH–H_2O, 11:9) yielded three frs (I–III). Fr. II consisted of **2** (7 mg). Compounds **4** (1.5 mg) and **5** (1.5 mg) were obtained from fr. III by semi-prep. HPLC on a CN column (*n*-hexane–*iso*-PrOH, 9:1). Purification of fr. 3 (75 mg) by semi-prep. HPLC (RP-18, MeOH–H_2O, 11:9) afforded **3** (3 mg).

1,7-*Dihydroxy*-3,4-*dimethoxyxanthone* (**1**). Yellow powder, mp 270–278° sublimed. UV λ_{max}^{MeOH} nm (log ε): 233 (4.28), 264 (4.40), 309 (3.85), 385 (3.63); ($AlCl_3$) 233, 280, 325, 445; ($AlCl_3$ + HCl) 232, 280, 325, 439; (NaOMe) 246, 269, 417; (NaOAc) 264, 309, 385; (NaOAc + H_3BO_3) 264, 308, 386. EIMS *m/z* (rel. int.): 288 $[M]^+$ (49), 273 (100), 245 (32). ^{1}H NMR (200.6 MHz, $CDCl_3$–CD_3OD, 20:1): δ7.49 (1H, *d*, *J* = 2.9 Hz, H-8), 7.25 (1H, *dd*, *J* = 9.1, 2.9 Hz, H-6), 6.42 (1H, *d*, *J* = 9.1 Hz, H-5), 6.37 (1H, *s*, H-2), 3.93 (3H, *s*, MeO-3), 3.89 (3H, *s*, MeO-4). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ180.3 (C-9), 159.6 (C-3), 158.0 (C-1), 154.0 (C-7), 149.1 (C-4b), 148.8 (C-4a), 128.1 (C-4), 124.7 (C-6), 120.1 (C-8a), 119.1 (C-5), 108.0 (C-8), 102.2 (C-9a), 94.7 (C-2), 60.8 (MeO-4), 56.5 (MeO-3).

1,7-*Dihydroxy*-2,3,4-*trimethoxyxanthone* (**2**). Yellow powder, mp 162–165°. UV λ_{max}^{MeOH} nm (log ε): 235 (4.33), 268 (4.40), 303 (3.90), 392 (3.64); ($AlCl_3$) 235, 285, 326, 454; ($AlCl_3$ + HCl) 235, 258, 284, 324, 449; (NaOMe) 249, 267, 278, 427; (NaOAc) 269, 390; (NaOAc + H_3BO_3) 269, 303, 351, 390. EIMS *m/z* (rel. int.): 318 $[M]^+$ (57), 303 (100), 288 (17), 275 (12), 289 (3); ^{1}H NMR (200.6 MHz, DMSO-d_6): δ12.62 (1H, *s*, OH-1), 10.00 (1H, *br s*, OH-7), 7.58 (1H, *d*, *J* = 9.0 Hz, H-5), 7.44 (1H, *d*, *J* = 3.0 Hz, H-8), 7.34 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-6), 4.06, 3.89, 3.83 (3H each, *s*, 3 × MeO). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ180.8 (C-9), 153.9 (C-7), 153.6 (C-3), 149.4 (C-1), 149.2 (C-4b), 145.8 (C-4a), 134.5 (C-2), 132.1 (C-4), 125.1 (C-6), 119.8 (C-8a), 119.3 (C-5), 107.7 (C-8), 104.1 (C-9a), 61.6, 61.4, 60.5 (3 × MeO).

5,7,4′-*Trihydroxy*-6,8-*dimethoxyisoflavone* (**3**). Amorphous powder, mp 148–151°. UV λ_{max}^{MeOH} nm (log ε): 271 (4.26), 335sh; ($AlCl_3$) 280, 320sh; ($AlCl_3$ + HCl) 281, 320sh; (NaOMe) 283, 330 sh; (NaOAc) 282, 340; (NaOAc + H_3BO_3) 272. EIMS *m/z* (rel. int.): 330 $[M]^+$ (87), 315 $[M-15]^+$ (100), 300 $[M-30]^+$ (12), 287 $[M-43]^+$ (58), 272 $[M-58]^+$ (29), 169 $[A_1-43]^+$ (15), 118 $[B_1]^+$ (17). ^{1}H NMR (200.6 MHz, DMSO-d_6): δ12.78 (1H, *s*, OH-5), 10.40 (1H, *br s*, OH-7), 9.55 (1H, *br s*, OH-4′), 8.40 (1H, *s*, H-2), 7.38 (2H, *d*, *J* = 8.5 Hz, H-2′ and H-6′), 6.82 (2H, *d*, *J* = 8.5 Hz, H-3′ and H-5′), 3.79, 3.80 (3H each, *s*, 2 × MeO). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ180.6 (C-4), 157.3 (C-4′), 153.9 (C-2), 150.7 (C-7), 148.7 (C-9), 145.7 (C-5), 131.5 (C-6), 130.0 (C-2′ and C-6′), 127.5 (C-8), 121.7[a] (C-3), 121.1[a] (C-1′), 115.0 (C-3′ and C-5′), 103.7 (C-10), 61.0, 60.0 (2 × MeO). [a]Assignments interchangeable.

Acetylation of compound **3**. Treatment of **3** (2 mg) with pyridine–Ac_2O (1:1) at room temp. afforded triacetate **3a** (2.5 mg). Amorphous powder, mp 95–98°. UV λ_{max}^{MeOH} nm (log ε): 204 (4.38), 257 (4.38). ^{1}H NMR (200.6 MHz, $CDCl_3$): δ7.90 (1H, *s*, H-2), 7.49 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 7.15 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 4.06, 3.96 (3H each, *s*, 2 × MeO), 2.46, 2.32, 2.10 (3H each, *s*, 3 × AcO).

5,7-*Dihydroxy*-6,8,4′-*trimethoxyisoflavone* (**4**). Yellow powder, mp 88–91° UV λ_{max}^{MeOH} nm (log ε): 270 (4.20); (NaOMe) 279, 345sh; ($AlCl_3$) 282; ($AlCl_3$ + HCl) 282; (NaOAc) 282, 341sh; (NaOAc + H_3BO_3) 273. EIMS *m/z* (rel. int.): 344 $[M]^+$ (41), 329 $[M-15]^+$ (79), 301 $[M-43]^+$ (30), 169 $[A_1-43]^+$ (1.4), 132 $[B_1]^+$ (8), 117 $[B_1-15]^+$ (7). ^{1}H NMR (200.6 MHz, $CDCl_3$): δ7.93 (1H, *s*, H-2), 7.45 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.98 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 4.03, 3.97, 3.84 (3H each, *s*, 3 × MeO). ^{1}H NMR (200.6 MHz, DMSO-d_6): δ12.75 (1H, *s*, OH-5), 10.75 (1H, *br s*, OH-7), 8.44 (1H, *s*, H-2), 7.50 (2H, *d*, *J* = 8.6 Hz, H-2′ and H-6′), 7.00 (2H, *d*, *J* = 8.6 Hz, H-3′ and H-5′), 4.00, 3.97, 3.78 (3H each, *s*, 3 × MeO).

5,7-*Dihydroxy*-8,4′-*dimethoxyisoflavone* (**5**). Yellow powder, mp 141–149°. UV λ_{max}^{MeOH} nm (log ε): 265 (3.90), 334sh; (NaOMe) 279, 334; ($AlCl_3$) 280; ($AlCl_3$ + HCl) 279; (NaOAc) 281, 333sh; (NaOAc + H_3BO_3) 266. EIMS *m/z* (rel. int.): 314 $[M]^+$ (64), 299 $[M-15]^+$ (100), 271 $[M-43]^+$ (64), 167 $[A_1-15]^+$ (2.3), 139 $[A_1-43]^+$ (16), 132 $[B_1]^+$ (8). ^{1}H NMR (200.6 MHz, $CDCl_3$): δ7.91 (1H, *s*, H-2), 7.45 (2H, *d*, *J* = 8.9 Hz, H-2′ and H-6′), 6.98 (2H, *d*, *J* = 8.9 Hz, H-3′ and H-5′), 6.44 (1H, *s*, H-6), 3.97, 3.84 (3H each, *s*, MeO-8, 4′). ^{1}H NMR (200.6 MHz, DMSO-d_6): δ12.59 (1H, *s*, OH-5), 10.40 (1H, *br s*, OH-7), 8.45 (1H, *s*, H-2), 7.50 (2H, *d*, *J* = 8.6 Hz, H-2′ and H-6′), 7.00 (2H, *d*, *J* = 8.6 Hz, H-3′ and H-5′), 6.32 (1H, *s*, H-6), 4.00, 3.79 (3H each, *s*, 2 × MeO).

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