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Homoisoflavanones from *Pseudoprospero firmifolium* of the monotypic tribe Pseudoprospereae (Hyacinthaceae: Hyacinthoideae)

Chantal Koorbanally [a], Sarisha Sewjee [a], Dulcie A. Mulholland [a,b,*], Neil R. Crouch [a,c], Anthony Dold [d]

[a] *Natural Products Research Group, School of Chemistry, University of KwaZulu-Natal, Durban 4041, South Africa*
[b] *School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, United Kingdom*
[c] *Ethnobotany Unit, South African National Biodiversity Institute, P.O. Box 52099, Berea Road 4007, Durban, South Africa*
[d] *Selmar Schonland Herbarium, Rhodes University, Grahamstown 6139, South Africa*

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Abstract

Five 3-hydroxy-type homoisoflavonoids, 3,5-dihydroxy-7,8-dimethoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone, 3,5-dihydroxy-7-methoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone, 3,5-dihydroxy-7,8-dimethoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone, 3,5,6-trihydroxy-7-methoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone and 3,5,7-trihydroxy-3-(3′-hydroxy-4′methoxybenzyl)-4-chromanone in addition to the nortriterpenoid, 15-deoxoeucosterol, have been isolated from the dichloromethane extract of the bulbs of *Pseudoprospero firmifolium*, the sole representative of the tribe Pseudoprospereae of the subfamily Hyacinthoideae of the Hyacinthaceae.

Keywords: *Pseudoprospero*, Hyacinthaceae; Homoisoflavonoid; Nortriterpenoid; 3,5-Dihydroxy-7,8-dimethoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone; 3,5-Dihydroxy-7-methoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone; 3,5-Dihydroxy-7,8-dimethoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone; 3,5,6-Trihydroxy-7-methoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone; 3,5,7-Trihydroxy-3-(3′-hydroxy-4′methoxybenzyl)-4-chromanone; 15-Deoxoeucosterol

1. Introduction

The sub-Saharan Hyacinthaceae comprises three subfamilies, the largest of which is the Hyacinthoideae with approximately 200 regional representatives. Until very recently, all such subfamily members were thought to belong to the tribe Massonieae; however, recent molecular phylogenetic analyses (Manning et al., 2004) revealed that the tribe Massonieae is only monophyletic when one taxon was excluded. This species, *Pseudoprospero firmifolium* (Baker) Speta, was accordingly transferred to its own tribe Pseudoprospereae J.C. Manning & Goldblatt. Historically, *P. firmifolium* had previously been included in the genus *Scilla* L. *s.l.* (as *Scilla firmifolia* Baker) before *Scilla* was shown to be restricted to the northern hemisphere and all southern hemisphere representatives subsequently placed in four genera, one of which was the monotypic *Pseudoprospero* Speta (1998). The current investigation sought to chemically profile this newly circumscribed tribe and to consider whether chemotaxonomic support for its establishment is evident.

2. Results and discussion

The air-dried and macerated bulbs of *Pseudoprospero* were extracted using dichloromethane and methanol. From the dichloromethane extract, five novel homoisoflavonoids

* Corresponding author. Address: School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, United Kingdom. Tel.: +44 1483 686827; fax: +44 1483 686851.
E-mail address: d.mulholland@surrey.ac.uk (D.A. Mulholland).

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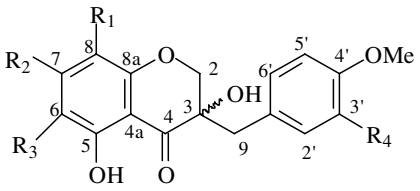

	R_1	R_2	R_3	R_4
1	OMe	OMe	H	OMe
2	H	OMe	H	OMe
3	OMe	OMe	H	OH
4	H	OMe	OH	OH
5	H	OH	H	OH

Fig. 1. Structures of compounds **1**–**5**.

of the 3-hydroxy-3-benzyl-4-chromanone type were isolated as well as the known spirocyclic nortriterpenoid, 15-deoxoeucosterol, previously isolated from *Scilla scilloides* Druce (Sholichin et al., 1982). No compounds of interest were isolated from the methanol extract.

The present finding of five homoisoflavonoids (see Fig. 1) and a spirocyclic nortriterpenoid in *P. firmifolium* is consistent with the known chemistry of the Hyacinthoideae (Pohl et al., 2000). Whereas the five novelties may presently be considered chemical markers for the tribe Pseudoprospereae, no significant new chemical subclasses were identified: both 3-hydroxy-3-benzyl-4-chromanones and nortriterpenoids are also known from tribe Massonieae and Hyacintheae.

High resolution mass spectrometry and 2D-NMR experiments were used to determine the structures of the compounds isolated and to assign all the ^{1}H and ^{13}C NMR resonances (Tables 1 and 2). Circular dichroism experiments were undertaken to confirm the stereochemistry at C-3 of the homoisoflavanones.

The HRMS of compound **1** showed a molecular ion peak at *m/z* 390.13076 (calculated 390.131468) which corresponded to the molecular formula $C_{20}H_{22}O_8$. The ^{1}H NMR spectrum of compound **1** showed the typical splitting pattern for a 3-hydroxy-3-benzyl-4-chromanone-type homoisoflavonoid with the 2H-2 resonances occurring as a pair of doublets (δ 4.08, 4.33; *d*, $J = 11.1$ Hz) and the 2H-9 resonances appearing superimposed at δ 2.95. The fully substituted resonance at δ 72.2 was assigned to C-3 and a hydroxy group was positioned here (Kirkiacharian et al., 1984). The ^{1}H NMR spectrum showed four methoxy group proton singlet resonances at δ 3.79, δ 3.81, δ 3.83 and δ 3.89, a single proton resonance at δ 6.13 attributed to a proton on the A-ring, and resonances ascribed to H-2′ (δ 6.66, *d*, $J = 1.98$ Hz), H-5′ (δ 6.76, *d*, $J = 8.13$ Hz) and H-6′ (δ 6.69, *dd*, $J = 1.98$, 8.13 Hz) of ring B.

Table 2
^{13}C NMR data for compounds **1**–**5** ($CDCl_3$, 100 MHz)

No.	Cpd 1	Cpd 2	Cpd 3	Cpd 4	Cpd 5
2	72.3	71.9	72.0	72.4	72.1
3	72.2	72.2	72.2	72.1	71.7
4	198.2	198.0	198.3	198.4	198.1
4a	100.2	100.5	100.2	100.3	100.5
5	159.7	163.9	159.8	157.1*	164.1
6	93.4	95.3	93.4	126.3	97.0
7	162.1	168.6	162.1	156.5*	165.1
8	126.3	94.5	129.7	92.9	95.6
8a	153.2	162.6	153.3	145.5	163.0
9	41.3	41.2	40.9	40.7	40.8
1′	126.3	126.5	127.0	127.0	127.1
2′	113.5	113.5	116.6	116.7	116.6
3′	148.5	148.6	145.8	145.6	145.2
4′	148.2	148.2	145.3	145.4	145.8
5′	110.8	110.8	110.4	110.4	110.4
6′	122.6	122.6	122.0	122.1	122.1
6-OMe					
7-OMe	56.3	55.8	56.3	56.5	
8-OMe	61.3		61.4		
3′-OMe	55.7	55.8			
4′-OMe	55.7	55.9	55.8	55.8	55.9

* Interchangeable.

Table 1
^{1}H NMR data for compounds **1**–**5** ($CDCl_3$, 400 MHz)

No.	Cpd 1	Cpd 2	Cpd 3	Cpd 4	Cpd 5
2	4.08 *d* (11.11)	4.06 *d* (11.11)	4.08 *d* (11.11)	4.08 *d* (11.11)	4.03 *d* (11.11)
	4.33 *d* (11.11)	4.20 *d* (11.11)	4.35 *d* (11.11)	4.34 *d* (11.11)	4.20 *d* (11.11)
3	–	–	–	–	–
6	6.13 *s*	6.09 *d* (2.18)	6.14 *s*	–	6.02 *d* (2.38)
7	–	–	–	–	–
8	–	6.03 *d* (2.18)	–	6.17 *s*	5.97 *d* (2.20)
9	2.95	2.93	2.91	2.92	2.89
2′	6.66 *d* (1.98)	6.68 *d* (1.78)	6.77 *d* (2.18)	6.79 *d* (2.18)	6.79 *d* (2.01)
5′	6.76 *d* (8.13)	6.78 *d* (8.13)	6.75 *d* (8.13)	6.75 *d* (8.13)	6.76 *d* (8.13)
6′	6.69 *dd* (1.98; 8.13)	6.71 *dd* (1.78; 8.13)	6.63 *dd* (1.98; 8.13)	6.65 *dd* (2.18; 8.13)	6.64 *dd* (2.01; 8.13)
5-OH	11.17 *s*	11.21 *s*	11.19 *s*	10.91 *s*	11.25 *s*
6-OMe	–	–	–	–	–
7-OMe	3.89 *s*	3.82 *s*	3.90 *s*	3.94 *s*	–
8-OMe	3.79 *s*	–	3.80 *s*	–	–
3′-OMe	3.81 *s*	3.82 *s*	–	–	–
4′-OMe	3.83 *s*	3.84 *s*	3.85 *s*	3.85 *s*	3.85 *s*
3′-OH	–	–	5.55 *s*	5.55 *s*	–
8-OH	–	–	–		–

Correlations seen in the NOESY spectrum between the H-2′ and H-5′ resonances and methoxy group proton resonances at δ 3.81 and δ 3.83, respectively, enabled placement of methoxy groups at C-3′ and C-4′. A hydrogen-bonded hydroxyl group proton resonance occurred at δ 11.17 in the ^{1}H NMR spectrum, indicating the presence of a hydroxyl group at C-5. This assignment was confirmed by the downfield chemical shift of δ 198.2 for the C-4 resonance. When there is a hydroxy group at the C-5 position, the carbonyl resonance occurs downfield, close to δ 200, due to hydrogen bonding between the hydroxyl group proton and the carbonyl group oxygen, whereas when a methoxy group is present at C-5, the carbonyl carbon resonance occurs typically around δ 192–196 (Adinolfi et al., 1986). The C-5 hydroxy group proton resonance showed a correlation in the NOESY spectrum with the A ring proton singlet at δ 6.13, enabling its assignment as H-6. This resonance (H-6), in turn, showed a correlation with the methoxy group proton resonance at δ 3.89 enabling the placement of this methoxy group at position C-7. The remaining methoxy group was placed at C-8. Additional confirmation of the structure was provided by a correlation seen in the HMBC spectrum between the C-6 methine carbon resonance (δ 93.4) and the C-5 hydroxy group proton resonance. Hence the structure of compound **1** was confirmed to be 3,5-dihydroxy-7,8-dimethoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone.

The ^{1}H NMR spectrum of compound **2** differed from that of compound **1** in having three methoxy groups, two of which could be placed at C-3′ and C-4′ as in compound **1**, a chelated hydroxyl group proton resonance at δ 11.21 (5-OH) and a pair of ring A *meta*-coupled proton resonances at δ 6.09 and δ 6.03. The 5-OH proton resonance was seen to correlate with the resonance at δ 6.09 in the NOESY spectrum enabling its assignment as H-6, and thus the resonance at δ 6.03 was assigned as H-8. The remaining methoxy group resonance at δ 3.82 showed correlations with both these resonances, and was placed at C-7. Hence the structure of compound **2** was determined to be 3,5-dihydroxy-7-methoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone.

Compound **3** differed from compound **1** in that it had a hydroxy group at C-3′ instead of a methoxy group. The NOESY spectrum showed a correlation between the H-5′ proton resonance and the methoxy group proton resonance at δ 3.85, but the H-2′ resonance showed no such correlation. The HMBC spectrum showed correlations between the C-2′ and C-4′ resonances and a phenolic proton resonance at δ 5.55. Hence, compound **3** was found to be the novel 3,5-dihydroxy-7,8-dimethoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone.

Compound **4** was found to be 3,5,6-trihydroxy-7-methoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone. A chelated 5-OH group proton resonance (δ 10.91, s), two methoxy group proton resonances (δ 3.94, δ 3.85), an ABX ring B system (δ 6.79, *d*, $J = 2.18$, H-2′; δ 6.75, *d*, $J = 8.13$, H-5′; δ 6.65, *dd*, $J = 2.18$, 8.3, H-6′) and a single ring A proton resonance (δ 6.17) were observed in the ^{1}H NMR spectrum. The methoxy group proton resonance at δ 3.85 showed a correlation in the NOESY spectrum with the H-5′ resonance and was placed at C-4′ with a hydroxyl group placed at C-3′ as in compound **3**. The second methoxy group proton resonance showed a correlation with the A ring proton and as a result of this, and the fact that an oxygenated substituent is required at C-7 on biosynthetic grounds, the methoxy group was placed at C-7. No correlation was seen between the ring A proton and the 5-hydroxy group proton resonance, hence a hydroxyl group was placed at C-6 and the single A ring proton at H-8. The ^{13}C chemical shifts of 5,6-dihydroxy-7-methoxy- and 5,8-dihydroxy-7-methoxy- substituted ring A homoisoflavanones have been compared (Koorbanally et al., 2006) and values for compound **4** confirm the presence of the former arrangement of substituents.

Compound **5** was identified as 3,5,7-trihydroxy-3-(3′-hydroxy-4′methoxybenzyl)-4-chromanone. The single methoxy group was placed at C-4′ due to a correlation seen in the NOESY spectrum with the H-5′ resonance (δ 6.76, *d*, $J = 8.13$ Hz). As in compound **2**, the 5-OH phenolic proton resonance was present at δ 11.25, a pair of *meta*-coupled proton resonances at δ 6.02 and δ 5.97 were assigned as H-6 and H-8, respectively, and a hydroxyl group was placed at C-7.

Circular dichroism experiments showed negative peaks at around 290 nm and positive peaks at around 320 nm in compounds **1**–**5**, indicating that these compounds were racemic. (Adinolfi et al., 1988).

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in $CDCl_3$ on a 400 MHz Varian spectrometer. HRMS were recorded on a Thermo Finnigan Mat 95 mass spectrometer. Ultraviolet absorption spectra were obtained on a Varian DMS 300 UV–Vis spectrophotometer and Infrared spectra were recorded using a Nicolet Impact 400D Fourier-transform infra-red (FT-IR) spectrometer. Optical rotations were measured at room temperature in methanol using a Perkin–Elmer Polarimeter – Model 341.

3.2. Plant material and extraction

Bulbs of *P. firmifolium* (Baker) Speta (595.44 g) were collected at Howison's Poort in the Grahamstown District, Eastern Cape Province, South Africa and a voucher (*Dold 4698*, GRA) lodged for verification purposes. The plant material was air-dried, chopped and extracted on a Labcon Mechanical shaker with 2 L of dichloromethane and methanol, respectively. The solvent was reduced under pressure to yield a 7.37 g dichloromethane extract and 35.16 g methanol extract.

3.3. Chromatography

The dichloromethane and methanol extracts of *P. firmifolium* were fractionated using a CH_2Cl_2:EtOAc and CH_2Cl_2:MeOH step gradient, respectively. No compounds of interest were isolated from the methanol extract. The dichloromethane extract (7.37 g) was separated using silica gel (Merck 9385) and repeated column chromatography afforded compound **1**, 3,5-dihydroxy-7,8-dimethoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone (14.4 mg), compound **2**, 3,5-dihydroxy-7-methoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone (13.6 mg), compound **3**, 3,5-dihydroxy-7,8-dimethoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone (10.6 mg), compound **4**, 3,5,6-trihydroxy-7-methoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone (10.2 mg), compound **5**, 3,5,7-trihydroxy-3-(3′-hydroxy-4′methoxybenzyl)-4-chromanone (17.6 mg) and 15-deoxoeucosterol (15.2 mg) (Sholichin et al., 1982).

Compound **1**: *3,5-dihydroxy-7,8-dimethoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone*. 14.4 mg, yellow amorphous compound. λ_{max} nm ($\log\varepsilon$): 290 (2.26). $[\alpha]_D^{25} = -131.6$ ($c = 0.019$ g/100 mL). IR λ_{max} (NaCl) cm^{-1}: 3354(–OH), 2920, 1630(C=O), 1264, 1138. HRMS [M^+] at *m/z* 390.13076, $C_{20}H_{22}O_8$ requires 390.131468.

Compound **2**: *3,5-dihydroxy-7-methoxy-3-(3′,4′-dimethoxybenzyl)-4-chromanone*. 13.6 mg, yellow amorphous gum. λ_{max} nm ($\log\varepsilon$): 289 (2.28). $[\alpha]_D^{25} = -86.11$ ($c = 0.018$ g/100 mL). IR λ_{max} (NaCl) cm^{-1}: 3471(–OH), 2928, 1631(C=O), 1260, 1157. HRMS [M^+] at *m/z* 376.1153, $C_{19}H_{20}O_8$ requires 376.115818.

Compound **3**: *3,5-dihydroxy-7,8-dimethoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone*. 10.6 mg, yellow gum. λ_{max} nm ($\log\varepsilon$): 291 (2.46). $[\alpha]_D^{25} = -75.00$ ($c = 0.012$ g/100 mL). IR λ_{max} (NaCl) cm^{-1}: 3431(–OH), 2931, 1634(C=O), 1211, 1017. HRMS [M^+] at *m/z* 376. 1153, $C_{19}H_{20}O_8$ requires 376.115818.

Compound **4**: *3,5,6-trihydroxy-7-methoxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone*. 10.2 mg, pale yellow gum. λ_{max} nm ($\log\varepsilon$): 293 (2.42). $[\alpha]_D^{25} = -38.46$ ($c = 0.013$ g/100 mL). IR λ_{max} (NaCl) cm^{-1}: 3412(–OH), 2958, 1648(C=O), 1270, 1131. HRMS [M^+] at *m/z* 362.1000, $C_{18}H_{18}O_8$ requires 362.100168.

Compound **5**. 17.6 mg, yellow amorphous compound. λ_{max} nm ($\log\varepsilon$): 290 (2.42). $[\alpha]_D^{25} = -76.92$ ($c = 0.013$ g/100 mL). IR λ_{max} (NaCl) cm^{-1}: 3396(–OH), 2925, 1639(C=O), 1275, 1029. HRMS [M^+] at *m/z* 332.08947, $C_{17}H_{16}O_7$ requires 332.089603.

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