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Homoisoflavonoids and stilbenes from the bulbs of *Scilla nervosa subsp. rigidifolia*

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Abstract

Thirteen homoisoflavonoids, nine of which are new: 3-(4-methoxybenzyl)-5,7-dimethoxychroman-4-one, 3-(4-hydroxy-3-methoxybenzyl)-5-hydroxy-7-methoxychroman-4-one, 3-(4-methoxybenzylidene)-5,7-dihydroxy-6-methoxychroman-4-one, 3-(4-hydroxybenzylidene)-5-hydroxy-7-methoxychroman-4-one, 3-(4-hydroxy-3-methoxybenzyl)-5-hydroxy-6,7-dimethoxychroman-4-one, 3-(3,4-dimethoxybenzyl)-5,7-dihydroxychroman-4-one, 3-(4-methoxybenzyl)-6-hydroxy-5,7-dimethoxychroman-4-one, 3-(4-hydroxybenzyl)-5,6,7-trimethoxychroman-4-one and 3-(4-methoxybenzyl)-8-hydroxy-5,7-dimethoxychroman-4-one, were isolated from the bulbs of *Scilla nervosa* together with four known ones and three known stilbene derivatives. The structures of these secondary metabolites were characterized by spectroscopic means and by comparison with published information for known compounds.

Keywords: Scilla nervosa; Hyacinthaceae; Bulbs; Benzylchroman-4-ones; Benzylidenechroman-4-ones; Homoisoflavonoids; Stilbene derivatives

1. Introduction

The genus *Scilla* is believed to globally represent 80 taxa. Four species occur in southern Africa of which *Scilla nervosa* is the only member known to occur in Botswana (Barnes, Turton & Kalake, 1994; Hutchings, 1996). In continuation of our studies on marketed plants of Eastern and Southern Africa (Abegaz, 1996; Bezabih, Motlhagodi & Abegaz, 1997; Ngadjui, Dongo, Happi, Bezabih & Abegaz, 1998) we have investigated the bulbs of *S. nervosa* (Burch.) Jessop subspecies *rigidifolia* (Hyacinthaceae). This plant is important in Zulu medicine and is used to treat pains associated with rheumatic fever and as purges for children. In Botswana the plant is alleged to enhance female fertility and to treat infections. There are no published reports dealing with the chemical constituents of this plant. In this paper we present the results of our studies on the bulbs of this plant which has resulted in the isolation and characterization of thirteen homoisoflavonoids (**1**–**13**) of which nine (**2**, **4**–**6**, **8**, **10** and **11**–**13**) are reported here for the first time. Three known stilbene derivatives (**14**–**16**) were also identified.

2. Results and discussion

The 20% methanol in chloroform soluble portion of the organic extract of the bulbs of *S. nervosa* was subjected to flash chromatography followed by further CC and PTLC purification to yield the four 3-benzylidenechroman-4-ones (**1**, **2**, **3** and **4**), nine 3-benzylchroman-4-ones (**5**, **6**–**13**) and three stilbene derivatives (**14**–**16**).

*2.1. Structure elucidation of 3-benzylidenechroman-4-ones **1**–**4***

The EIMS and ^{13}C NMR/DEPT spectra of these compounds led to the molecular formulas of $C_{17}H_{14}O_6$ for **1**, $C_{18}H_{16}O_6$ for **2**, $C_{16}H_{12}O_5$ for **3** and $C_{17}H_{14}O_5$

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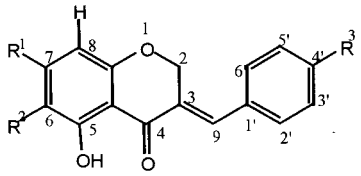
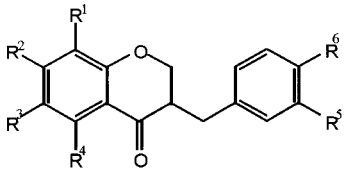
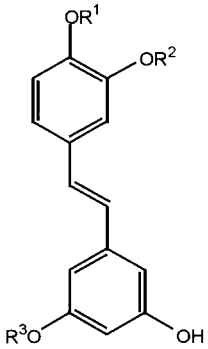


	R^1	R^2	R^3
1	HO	MeO	HO
2	HO	MeO	MeO
3	HO	H	HO
4	MeO	H	HO



	R^1	R^2	R^3	R^4	R^5	R^6
5	H	MeO	H	MeO	H	MeO
6	H	MeO	H	HO	MeO	HO
7	H	HO	H	HO	HO	MeO
8	H	MeO	MeO	HO	MeO	HO
9	H	HO	MeO	HO	H	HO
10	H	HO	H	HO	MeO	MeO
11	H	MeO	HO	MeO	H	MeO
12	H	MeO	MeO	MeO	H	HO
13	HO	MeO	H	MeO	H	MeO



	R^1	R^2	R^3
14	H	Me	Me
15	Me	H	H
16	H	Me	H

for **4**. The 3-benzylidenechroman-4-one system was established from analyses of their ^{1}H NMR spectra and the E-geometry of the $\Delta^{3,9}$ was clearly discerned by the characteristic ^4J-coupling of the C-2 and C-9 protons and the downfield chemical shift of the C-9 proton at δ 7.6–7.9. The Z-geometry places the C-9 proton away from the anisotropic region of the carbonyl group and makes this vinyl proton to resonate at a higher field (ca δ 5.5) (Heller & Thamm, 1981), which was not observed for any of the compounds in this study. All the four compounds also showed resonance signals typical of an AA′BB′ system for ring B protons and a one proton, low field (ca δ 13) signal that is appropriate to a chelated 5-OH group. Comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 3) of **1** and **2** suggested that these two compounds have the same degree of substitution in ring A. Each has a free hydroxyl group at C-7 (UV- bathochromic shift with NaOAc). The ^{1}H NMR (Table 1) of **2** showed a sharp singlet at δ 6.03 while its ^{13}C NMR displayed two methoxy carbon resonances at δ_C 61.2 and 55.7, one of which should be *ortho*, *ortho* disubstituted because of the downfield chemical shift at δ_C 61.2 (Table 3).

Table 1
^{1}H NMR spectral data of 3-benzylidenechroman-4-ones **1**, **3**, **5**, and **7** in CD_3COCD_3 and **2** and **6** in $CDCl_3$ (multiplicities and J values are given in Hz in parenthesis)

	1	**2**	**3**	**4**
H-2a	5.41 (d, 1.8)	5.28 (br s)	5.43 (d, 2.1)	5.42 (d, 2.1)
H-2b	5.41 (d, 1.8)	5.28 (br s)	5.43 (d, 2.1)	–
H-3	–	–	–	–
H-6	–	–	6.01 (d, 2.1)	6.00 (d, 2.1)
H-8	6.00 (s)	6.03 (s)	5.95 (d, 2.1)	6.06 (d, 2.1)
H-9a	7.79 (d, 1.5)	7.80 (d, 1.0)	7.78 (d, 1.5)	7.76 (br s)
H-9b	7.79 (d, 1.5)	7.80 (d, 1.0)	7.78 (d, 1.5)	–
H-2′	7.40 (d, 8.9)	7.25 (d, 8.7)	7.41 (d, 8.7)	7.37 (d, 8.8)
H-3′	7.02 (d, 8.9)	6.97 (d, 8.7)	7.02 (d, 8.7)	6.98 (d, 8.8)
H-5′	7.02 (d, 8.9)	6.97 (d, 8.7)	7.02 (d, 8.7)	6.98 (d, 8.8)
H-6′	7.40 (d, 8.9)	7.25 (d, 8.7)	7.41 (d, 8.7)	7.37 (d, 8.8)
5-OH	13.06 (s)	12.93 (s)	12.92 (br s)	12.85 (s)
5-OMe	–	–	–	–
6-OMe	3.83 (s)	3.94[a]	–	–
7-OMe	–	–	–	3.86 (s)
8-OMe	–	–	–	–
3′-OMe	–	–	–	–
4′-OMe	–	3.86[a]	–	–

[a] Assignments in the same vertical column may be interchanged.

Table 2
^{1}H NMR spectral data of 3-benzylchroman-4-ones **4**, **9**, **10** in $CD_3CO\,CD_3$ and **8** and **11**–**13** in $CDCl_3$ (multiplicities and *J* values are given in Hz in parenthesis)

	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
H-2a	4.26 (dd, 11.1,4.2)	4.28 (dd, 11.4, 4.2)	4.32 (dd, 11.4, 4.8)	4.27 (dd, 11.4, 4.2)	4.29 (dd, 11.1, 4.5)	4.32 (dd, 11.4, 4.5)	4.24 (dd, 10.8, 3.3)	4.27 (dd, 11.1, 3.6)	4.35 (dd, 11.1, 3.9)
H-2b	4.05 (dd, 11.4,8.7)	4.12 (dd, 11.4, 7.2)	4.13 (dd, 11.7, 8.7)	4.11 (dd, 11.4, 7.2)	4.16 (dd, 11.4, 8.1)	4.14 (dd, 11.7, 8.4)	4.06 (dd, 10.5, 6.3)	4.09 (dd, 11.1, 6.9)	4.19 (dd, 11.1, 6.6)
H-3	2.72 m	2.83, m	2.94, m	2.82 (m)	2.91(m)	2.99 (m)	2.69 (m)	2.70 (m)	2.76 (m)
H-6	6.14 (d, 2.1)	6.06 (d, 2.1)	5.95 (d, 2.1)	–	–	5.91 (d, 2.1)	–	–	6.16 (s)
H-8	6.06 (d, 2.1)	5.97 (d, 2.1)	5.92 (d, 2.1)	6.02 (s)	5.95	5.94 (d, 2.1)	6.26 (s)	6.25 (s)	–
H-9a	3.11 (dd, 13.8, 4.5)	3.17 (dd, 13.7, 4.4)	3.12 (dd, 13.5, 4.8)	3.17 (dd, 13.8,4.2)	3.13 (dd, 13.8, 4.5)	3.18 (dd, 13.8, 4.5)	3.15 (dd, 12.9, 3.0)	3.14 (dd, 12.9, 3.3)	3.17 (dd, 13.5, 3.6)
H9b	2.56 (dd, 13.5, 10.2)	2.65 (dd, 13.8, 10.7)	2.64 (dd, 13.5, 9.9)	2.64 (dd, 13.5, 10.5)	2.67 (dd, 13.8, 10.2)	2.68 (dd, 13.8, 10.2)	2.65 (dd, 12.6, 10.7)	2.65(dd, 12.9, 10.5)	2.65 (dd, 13.2, 10.5)
H-2′	7.17 (d, 8.4)	6.81 (d, 2.1)	6.78 (d, 2.1)	6.81 (d, 2.1)	7.10 (d, 8.4)	6.92 (d, 1.8)	7.12 (d, 8.4)	7.06 (d, 8.4)	7.15 (d, 8.7)
H-3′	6.86 (d, 8.7)	–	–	–	6.80 (d, 8.7)	–	6.82 (d, 7.8)	6.80 (d, 8.7)	6.84 (d, 8.4)
H-5′	6.86 (d, 8.7)	6.80 (d, 8.0)	6.88 (d, 8.4)	6.80 (d, 8.1)	6.80 (d, 8.7)	6.88 (d, 8.1)	6.82 (d, 7.8)	6.80 (d, 8.7)	6.84 (d, 8.4)
H-6′	7.17 (d, 8.4)	6.71 (dd, 8.1, 2.2)	6.70 (dd, 8.4, 2.1)	6.70 (dd, 8.1, 2.1)	7.10 (d, 8.4)	6.79 (dd, 8.1, 2.1)	7.12 (d, 8.4)	7.06 (d, 8.4)	7.15 (d, 8.7)
5-OH	–	12.12 (s)	12.23 (s)	11.97 (s)	12.36 (s)	12.23 (s)	–	–	–
5-OMe	3.82[a]	–	–	–	–	–	3.90[a]	3.93[a]	3.96[a]
6-OMe	–	–	–	3.88[a]	3.76	–	–	3.81[a]	–
7-OMe	3.81[a]	3.90[a]	–	3.83[a]	–	–	3.88[a]	3.88[a]	3.90[a]
8-OMe	–	3.90–	–	–	–	–	–	–	–
3′-OMe	–	3.81[a]	–	3.80[a]	–	3.80[a]	–	–	–
4′-OMe	3.75[a]	–	3.81	–	–	3.78[a]	3.77[a]	–	3.78[a]

[a] Assignments in the same vertical column may be interchanged.

Table 3
^{13}C NMR spectra of homoisoflavonoids in CD_3COCD_3 (**1**, **3**, **5**, **7**, **9**, **10**) and in $CDCl_3$ (**2**, **6**, **8**, **11**, **12**, **13**)

	1	2	3	5	6	7	8	9	10	11	12	13
C-2	68.2	67.7	68.2	69.8	69.1	70.3	69.4	70.1	70.1	69.1	69.2	69.6
C-3	126.6	126.9	126.5	48.9	46.7	47.1	46.9	47.4	47.1	48.5	48.7	49.1
C-4	186.5	186.2	185.8	189.9	197.9	198.9	198.7	199.8	198.9	191.8	192.3	191.8
C-4a	104.0	103.6	103.1	106.0	102.7	102.7	102.8	102.9	102.7	108.0	108.7	105.5
C-5	158.6	155.5	166.2	165.4	164.5	165.6	161.0	156.7	167.4	158.3	154.6	158.4
C-6	127.9	127.6	97.1	93.9	95.0	96.9	130.2	129.7	96.9	133.9	137.5	89.5
C-7	160.1	161.1	167.6	166.2	167.8	167.4	159.0	159.9	165.6	157.2	159.7	155.5
C-8	95.3	94.3	95.6	93.3	93.9	95.6	91.4	95.3	95.6	96.0	96.2	127.5
C-8a	158.6	158.1	163.3	163.2	162.9	164.3	155.5	159.5	164.3	146.3	154.9	149.6
C-9	137.7	137.5	137.6	32.1	32.2	32.5	32.3	32.3	32.7	32.1	32.3	32.2
C-1′	130.9	128.7	127.8	131.5	131.1	132.0	131.1	130.0	131.7	130.4	130.1	130.5
C-2′	133.5	132.3	133.5	130.7	110.8	116.7	115.3	131.0	113.6	130.2	130.4	130.4
C-3′	116.7	114.5	116.7	114.5	145.6	147.5	145.6	116.2	149.1	114.1	115.7	114.2
C-4′	157.0	157.5	160.2	159.0	145.7	147.2	145.8	157.1	150.4	154.0	160.0	152.3
C-5′	116.7	114.5	116.7	114.5	115.2	112.5	111.0	116.2	112.7	114.1	115.7	114.2
C-6′	133.5	132.3	133.5	130.7	120.6	120.9	120.8	131.0	122.0	130.2	130.4	130.4
C5-OMe	–	–	–	56.0[a]	–	–	–	–	–	61.8	61.8[a]	56.5[a]
C6-OMe	60.7	61.2	–	–	–	–	61.1	60.8	–	–	61.5[a]	–
C7-OMe	–	–	–	55.8[a]	56.0[a]	–	56.4[a]	–	–	55.4[a]	56.3	55.5[a]
C8-OMe	–	–	–	–	–	–	–	–	–	–	–	–
C3′-OMe	–	–	–	–	55.7[a]	–	56.2[a]	–	56.1[a]	–	–	–
C4′-OMe	–	55.7	–	55.3[a]	–	56.3	–	–	56.0[a]	56.4[a]	–	56.4[a]

[a] Assignments in the same vertical column may be interchanged.

The structures **1** and **2** were proposed on the basis of the foregoing spectroscopic evidence. The spectroscopic and other physical data of **1** were found to be similar to those reported for autumnalin isolated from the bulbs of *Eucomis autumnalis* and *Colchicum doerfleri* (Dictionary of Natural Products on CD-ROM, 1998; Sidwell & Tamm, 1970). Compound **2** has not been reported before. An isomer of **2**, 4′-*O*-methylpunctatin, having the methoxy substituent at C-8 instead of at C-6, has been reported by Sidwell and Thamm in 1970. These authors report the ^1H NMR (100 MHz) spectral data in DMSO-d_6. The spectrum of **2** in $CDCl_3$ showed slight differences when compared with the reported spectra for 4′-*O*-methyl-punctatin in DMSO-d_6. These subtle differences persisted even after measuring the spectrum of **2** in DMSO-d_6, and it was not possible to establish unequivocally the difference in the structure of these two substances on the basis of ^1H NMR alone. Our decision to maintain that the two compounds are different stems from careful study of the ^{13}C NMR data and comparing the chemical shift values obtained for the compound with those of the many compounds isolated in this study and especially those of **1**, **8** and **9** and homoisoflavonoids of related structures in the literature (Mašterovā et al., 1991).

The spectral and physical data for compound **3** were found to be identical to those reported for 4′-demethyleucomin isolated from the bulbs of *Eucomis punctata* (Dictionary of Natural Products on CD-ROM, 1998). It was possible to conclude from the ^1H NMR spectrum (Table 1) of compound **4** that it was a monomethyl ether of compound **3**. The site of methylation for **4** was established to be at C-7 from the observed NaOAc induced bathochromic shift for **3** but not for **4**. The MS showed m/z 298 [M^+] and RDA fragments at m/z at 167 and 132. To the best of our knowledge compound **4** has not been reported previously, although, its isomer, eucomin, which has the methoxy group at C-4′ has been reported (Heller & Thamm, 1981).

2.2. Structure elucidation of 3-benzylchroman-4-ones (*5–13*)

This group of 3-benzylchroman-4-ones (**5–13**) is typically characterized by the appearance of two sets of double doublet signals (δ 4.3/4.1 and δ 3.1/2.6) for the two protons each on C-2 and C-9, respectively, and a prominent complex multiplet (δ 2.8) signal for the proton at C-3. The δ_C values for C-2, C-3 and C-9 were also strikingly consistent in all these compounds and appeared at ca δ 69 (t), δ 48 (d) and δ 32 (t), respectively (Table 3).

The ^1H NMR spectra (Tables 1 and 2) of **5**, **9** and **11–13** showed these compounds to have a 4′-oxygen substituted AA′BB′ system in ring B. This was also observed from their ^{13}C NMR spectra (Table 3). The most straightforward spectra to interpret was that of **5** which showed two *meta* coupled proton signals at δ

6.06 and 6.14 (Table 1) indicative of a 5,7-disubstituted ring A. Three methoxy signals were observed and these were located at the 5-,7- and 4′-positions. The methylation at C-5 is further supported by the relatively higher field of the C-4 carbonyl resonance at δ 189.9 (Table 3), compared to those that contain a free -OH at C-5. Although compound **5** has been utilized as an intermediate in the synthesis of eucomol (Heller & Thamm, 1981), this is the first report of its occurrence in nature.

The spectra of **9** and **11**–**13** showed that these compounds differ from **5** by having an additional oxygen substituent in ring A. Compound **9** showed spectral data very similar to those reported for eucomnalin, a compound isolated previously from *Eucomis autumnalis* (Tamm, 1972). The ^{13}C NMR data (Agrawal, 1989) is in complete agreement with the given structure. Analyses of the EIMS, ^{13}C NMR and DEPT data of compounds **11**–**13** led to the formulation of the same molecular formula, $C_{19}H_{20}O_6$, for all of them. The ^{1}H NMR spectra of **11** and **13** showed three methoxy signals each and a sharp singlet (1H) at δ 6.26 for **11** and δ 6.16 for **13**. Since neither compound **11** nor **13** showed signals assignable to a chelated OH group it was concluded that the C-5 positions in both compounds were occupied by methoxy groups. Also, since the UV spectra of both compounds (Experimental) were not affected by the introduction of shift reagents ($AlCl_3$ and NaOAc), it was assumed that the C-7 positions of both contain OMe groups. The chemical shifts of the methoxy carbons were observed to be at δ_C 61.8, 55.4 and 56.4 for **11**, and δ_C 55.5, 56.4 and 56.5 for **13**. These values can only be accounted for by assigning the location of the methoxy groups as shown. Both **11** and **13** are reported here for the first time. The ^{1}H NMR spectrum of **12** (Table 2) was similar to those of **11** and **13** and showed also a sharp singlet proton signal at δ 6.25 in addition to three methoxy signals. The structure of **12** was arrived at by similar reasoning as above, but taking into consideration the resonance positions of the methoxy carbons observed at δ_C, 56.3, 61.5 and 61.8. The UV spectrum of **12** was not affected by the introduction of NaOAc confirming the absence of free -OH group at C-7. The ^{13}C NMR (Table 3) and the EIMS (Experimental) data were in complete agreement with structure **12**. To the best of our knowledge **12** is reported here for the first time.

The ^{1}H NMR spectra of the homoisoflavanones (**6**, **7**, **8** and **10**) indicated that all of them contain a chelated OH group at the C-5 position ($\delta_H \approx 12$). This was also supported by the carbonyl resonance signals observed in the ^{13}C NMR of these compounds at ca δ 198 and also by UV spectral measurements which showed bathochromic shifts upon addition of aluminum chloride (Experimental). Their ^{1}H NMR spectra also contain ABC system of protons in ring B. This was confirmed by the ^{13}C NMR spectra (Table 3) of each compound which displayed two non-protonated carbon signals, between δ_C 145 and 150 characteristic of a 3′,4′-dioxygenated ring B.

The presence of two oxygen substitutents at the 5,7-positions of ring A of compounds **6**, **7** and **10** was established readily from the ^{1}H NMR spectra (Tables 1 and 2) which showed two *meta*-coupled signals were observed for each of the compounds. UV spectral investigations on all three compounds employing shift reagent, sodium acetate, (Experimental) suggested free C7-OH groups for **7** and **10**, but not for **6**. The ^{1}H NMR spectrum of **7** also showed the presence of a methoxy group whose location at C-4′ was determined by nOe measurements and observing the enhancement of the H-5′ signal at δ 6.88. Compound **6** was found to contain two methoxy groups and the site of one of them was determined to be at C-7 (from UV data, see above). The second methoxy signal which should be in ring B was assigned the C-3′ position by comparison with the proton spectrum of **7**. The 6′,2′,5′ protons of ring B constitute an ABC system, respectively, of protons in compound **7**. In compound **6**, where the 3′-OH is methylated instead of the 4′, the order of appearance of the ABC system changes to 6′,5′,2′. The ^{1}H NMR spectrum of **10** also showed two methoxy groups at δ 3.80 and 3.78, and in view of the already presented data above, it was concluded that these have to be at C-3′ and C-4′. Compound **7** has been isolated previously from *Muscari comosum* and the reported spectroscopic and physical data are in complete agreement with those determined for **7** in this study (Adinolfi et al., 1985). Compounds **6** and **10** are reported here for the first time.

The ^{1}H NMR spectrum of **8** (Table 2) showed a sharp singlet signal for 1H at δ 6.02, a set of three proton signals forming an ABC system at δ 6.70, 6.80 and 6.81 and three methoxy resonances. Its structure was established using similar reasoning as above, but taking into consideration the resonance positions of the three methoxy carbons at δ 56.2, 56.4 and 61.1 and also knowing from UV and ^{13}C NMR (Table 3) data that compound **8** is a 5-hydroxy-7-methoxy homoisoflavone. The ^{13}C NMR and the EIMS spectral data were in complete agreement with structure **8**. Compound **8** is also reported here for the first time.

The EIMS of the 3-benzylchroman-4-ones (**5**–**13**) showed the expected molecular ions. The most characteristic fragmentation was observed to be the cleavage of the 3–9 bond to give a benzylic cation, which in most cases was the base peak in the spectrum. RDA fragmentations, though with less intense ions, were also observed in all cases.

2.3. Structure elucidation of stilbenes (**14–16**)

Each of the ^{1}H NMR spectra of **14–16** was characterized by the presence of two vinyl proton signals with a *trans* geometry. The ^{13}C NMR spectra showed fourteen aryl/vinyl signals, and no C=O resonances. Detailed analysis of the spectra suggested that these compounds were stilbenes. The ^{1}H NMR of **14** showed the presence of eight aryl/vinyl proton resonances. Two of them form an AB system δ 6.97 and 7.10 (d, J = 16.2 Hz), the large coupling constant indicating a *trans* geometry. A set of three proton resonances at δ 7.02, 7.22 and 6.82, were assigned to a 1,3,4-trisubstituted phenyl group, while another set of three proton signals at δ 6.31, 6.62 and 6.63 were consistent with a 1,3,5-trisubstituted second phenyl group. The above data were consistent with a stilbene skeleton and structure **14** was assigned. The ^{13}C NMR of compound **14** was also found to be in full agreement with the assigned structure (Experimental). This compound has been isolated from the legume *Cassia didymobotrya* (Dictionary of Natural Products on CD-ROM, 1998). Compounds **15** and **16** were isolated as a mixture and their structures deduced from spectroscopic data (Experimental). Both **15** and **16** are known compounds and have been assigned the names rhapontigenin and isorhapontigenin, respectively (Dictionary of Natural Products on CD-ROM, 1998).

The finding of homoisoflavonoids is chemotaxonomically consistent with similar findings in many plants belonging to the Hyacinthaceae. This study identifies *Scilla nervosa* as a most suitable source for obtaining multigram quantities of **1**, **3** and **5**. The homoisoflavonoids found in this plant belong to the parent 3-benzylidenechroman-4-one and the dihydro-derivative, 3-benzylchroman-4-one skeletons. Although cardenolides are commonly reported from other *Scilla* species (Titel & Wagner, 1980) our detailed study on the organic extract of this plant did not reveal the presence of any. The presence of stilbene derivatives in *Scilla* is noteworthy since none has been reported from Hyacinthaceae to date.

3. Experimental

3.1. General

Mps: uncorr.; IR: KBr disk; ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) spectra were determined on a Varian Gemini 2000 spectrometer with residual solvent peaks used as internal reference standards.

3.2. Plant material

Scilla nervosa (Burch.) Jessop. was purchased from a vendor near the railway station in Gaborone and grown in the Experimental Garden of the Chemistry Department of the University of Botswana. A voucher specimen (SN 1977) is kept in the Herbarium of the National Museum. The identity of the plant was established by Dr. L. Turton in collaboration with Royal Botanic Gardens, KEW.

3.3. Extraction, isolation and characterization

The air dried and powdered bulbs of *Scilla nervosa* (1.4 kg) was soaked in the mixture of MeOH/CH_2Cl_2 (1:1, 6 l) for 24 h, followed by MeOH (3 l) for one h. Concentration of the combined organic extracts under reduced pressure (T < 40°C) gave a sticky brown residue (210 g) which was extracted with 20% MeOH in $CHCl_3$. Removal of the solvent left 65 g of residue which was submitted to flash CC using $CHCl_3$ and introducing an EtOAc gradient and subsequently MeOH gradient in EtOAc. Frs (45) each of 250 ml were collected. Frs. were monitored by TLC and those containing similar constituents (as judged by TLC and sometimes by NMR) were combined. Frs 1–2 (4 g), eluted with $CHCl_3$, contained mainly hydrocarbons and were not investigated further. Frs 3–5, also eluted with $CHCl_3$ gave an oil ($\approx$10 g) which was subjected to flash CC (silica gel, 100 g) and eluted with hexane/$CHCl_3$ (1:1) to give compound **5** as yellow oil, 8 g. One of the later fractions was found to contain a second component which was separated from **5** by PTLC to yield 10 mg of **6**. Frs 6–9 gave a residue ($\approx$7 g) which was put on to a column of silica gel (110 g) and eluted with hexane containing increasing amounts of $CHCl_3$. Frs. collected gave mixtures which were purified by several PTLC procedures employing $CHCl_3$ as developing solvent to furnish **2** (10 mg), **4** (5 mg), **8** (10 mg), **10** (200 mg), **13** (40 mg) and **14** (30 mg). Frs. 10–14, eluted with $CHCl_3$/EtOAc (9:1) gave $\approx$3 g of residue which by further CC (silica gel, 40 g) and elution with hexane/EtOAc gradient yielded **11** (100 mg). PTLC on fr 15 using solvent $CHCl_3$/EtOAc (9:1) led to the isolation of pure **12** (10 mg) and **7** (150 mg). Frs 16–17 (2 g) was recrystallized using $CH_2Cl_2$to give 120 mg of **9** as plates. Frs 18–25 eluted with $CHCl_3$/EtOAc (4:1) gave **1**, 4 g, and **3**, 2 g. The polar frs 26–45 (4 g) was subjected to several chromatographic (CC and PTLC) procedures to yield ultimately a mixture of **15** and **16** (50 mg). Known compounds **1**, **3**, **7**, **9** and **14**, **15** and 16 were identified using spectroscopic and other physical data and comparison with published information.

3.3.1. 3-(4-Hydroxybenzylidene)-5,7-dihydroxy-6-methoxychroman-4-one (**1**)

Yellow powder; m.p. 242–245°C; UV λ_{max}^{MeOH} nm (log ϵ): 198 (4.90), 358 (4.30); $\lambda_{max}^{MeOH+NaOAc}$ nm

(log ε):237 (5.10), 406 (3.80); IR ν_{max}^{KBr} cm^{-1}: 3426, 1651, 1580, 1510, 1468, 1415, 1325, 1260, 1162; EIMS *m/z* (rel. int.): 314 $[M]^+$ (100), 299 (25), 183 (11), 167 (50), 131 (27); ^1H NMR: Table 1; ^{13}C NMR: Table 3.

3.3.2. 3-(4-Methoxybenzylidene)-5,7-dihydroxy-6-methoxychroman-4-one (**2**)

Yellow gum; UV λ_{max}^{MeOH} nm (log ε): 204 (3.94), 351 (3.93), 362 (3.95); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε):203 (3.92), 261 (3.26), 333 (3.45), 398 (4.00); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 203 (3.96), 260 (3.40), 330 (3.38), 395 (4.06); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε):221 (4.39), 328 (3.61), 347 (3.63), 371 (3.82), 390 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3420 (-OH), 1640 (C=O), 1510, 1460, 1255, 1160, 835; EIMS *m/z* (rel. int.): 328 $[M^+]$ (100), 313 (36), 183 (18), 167 (60), 147 (58), 146 (56), 131 (22); ^1H NMR (DMSO-d_6) δ: 12.87 (1, br s, 5-OH), 7.71 (1H, br s, H-9), 7.43 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.05 (2H, d, J = 9.0 Hz, H-3′, H-5′), 5.91 (1H,br s, H-8), 5.31 (2H, d, J = 1.5 Hz, H-2), 3.83 (3H, s, OMe), 3.67 (3H, s, OMe), ^1H NMR; Table 1; ^{13}C NMR: Table 3.

3.3.3. 3-(4-Hydroxybenzylidene)-5,7-dihydroxychroman-4-one (**3**)

Yellow powder; m.p. 208–211°C; UV λ_{max}^{MeOH} nm (log ε): 197 (4.80), 361 (5.00); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 218 (5.10), 484 (5.20); IR ν_{max}^{KBr} cm^{-1}: 3420, 1642, 1590, 1515, 1462, 1368, 1270, 1152, 1080; EIMS *m/z* (rel. int.): 284 $[M]^+$ (100), 267 (4), 255 (16), 242 (5), 239 (100); ^1H NMR: Table 1; ^{13}C NMR: Table 3.

3.3.4. 3-(4-Hydroxybenzylidene)-5-hydroxy-7-methoxychroman-4-one (**4**)

Yellow gum; UV λ_{max}^{MeOH} nm (log ε): 203 (4.13), 360 (3.70); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε):204 (4.15), 330 sh (4.01), 395 (3.95); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 206 (4.79), 363 (3.80); IR ν_{max}^{KBr} cm: 3450 (-OH), 2920, 2840, 2365, 1640 (C=O), 1470, 1290, 1165; EIMS *m/z* (rel. int.): 298 $[M^+]$ (100), 223 (40), 197 (38), 196 (18), 181 (35), 168 (40), 167 (70), 157 (30), 132 (45); ^1H NMR: Table 2.

3.3.5. 3-(4-Methoxybenzyl)-5,7-dimethoxychroman-4-one (**5**)

Colorless gum, $[\alpha]_D^{25}$ −70.6 (MeOH, *c* 0.38); UV λ_{max}^{MeOH} nm (log ε): 212 (4.32), 225sh (4.30), 283 (4.31); UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 215 (4.30), 228 (4.23), 290 (4.34); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): no change; UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 211(4.36), 283 (4.30); $\lambda_{max}^{MeOH+MeONa}$ nm (log ε): no change; IR ν_{max}^{KBr} cm^{-1}: 2932, 1669, 1616, 1575, 1512, 1455, 1383, 1252, 1211, 1159, 1127, 1097, 1031, 963; EIMS *m/z* (rel. int.): 328 $[M]^+$ (100), 313 (17), 221 (18), 208 (15), 207 (38), 180 (42), 152 (40), 137 (20), 121 (78); ^1H NMR: Table 1; ^{13}C NMR: Table 3.

3.3.6. 3-(4-Hydroxy-3-methoxybenzyl)-5-hydroxy-7-methoxychroman-4-one (**6**)

Colorless oil; UV λ_{max}^{MeOH} nm (log ε): 202 (3.93), 288 (3.56); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 202 (3.94), 312 (3.52); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 203 (3.95), 312 (3.52); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 203 (4.12), 288 (3.57); $\lambda_{max}^{MeOH+MeONa}$ nm(log ε): 204 (4.14), 288 (3.95); IR ν_{max}^{KBr} cm^{-1}: 3425 (–OH), 2925, 1650 (C=O), 1575, 1515, 1460, 1380, 1270, 1185, 1160, 1070, 1025, 800; EIMS *m/z* (rel. int.): 330 $[M^+]$ (15), 329 $[M^+–H]$ (35), 328 (100), 313 (10); ^1H NMR: Table 1; ^{13}C NMR: Table 3.

3.3.7. 3-(3-Hydroxy-4-methoxybenzyl)-5,7-dihydroxychroman-4-one (**7**)

White powder; m.p.140–142°C; UV λ_{max}^{MeOH} nm (log ε): 200 (5.30), 289 (5.10) 320 (5.00); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 208 (5.00), 333 (5.10); IR ν_{max}^{KBr} cm^{-1}: 3450, 1640, 1510, 1455, 1385, 1270, 1160; EIMS *m/z* (rel. int.): 316 $[M]^+$ (99), 164 (5), 152 (5), 137 (100); ^1H NMR: Table 1; ^{13}C NMR: Table 3.

3.3.8. 3-(4-Hydroxy-3-methoxybenzyl)-5-hydroxy-6,7-dimethoxychroman-4-one (**8**)

Yellow oil. $[\alpha]_D^{25}$ −10.7 (MeOH, *c* 0.56); UV λ_{max}^{MeOH} nm (log ε): 212 (3.85), 288 (3.74); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 208 (3.82), 224 (3.82), 314 (3.63); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 209 (3.83), 224 (3.85), 315 (3.76); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 214 (3.97), 289 (3.74); IR ν_{max}^{KBr} cm^{-1}: 3450 (–OH), 2930, 2640, 2380, 1640, 1505, 1450, 1385, 1280, 1160, 1120, 1030; EIMS *m/z* (rel. int.): 360 $[M^+]$ (100), 308 (10), 307 (15), 223 (17), 197 (10) 137 (75); ^1H NMR: Table 2; ^{13}C NMR: Table 3.

3.3.9. 3-(4-Hydroxybenzyl)-5,7-dihydroxy-6-methoxychroman-4-one (**9**)

Yellow plates from CH_2Cl_2; m.p. 198–200°C; UV λ_{max}^{MeOH} nm (log ε): 212 (4.39), 290 (4.25) 320 (5.00); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 204 (4.36), 222 (4.36), 297 (4.13), 331 (4.10); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): no change $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 223 (4.80), 328 (4.45); IR ν_{max}^{KBr} cm^{-1}: 3347, 1634, 1600, 1507, 1446, 1393, 1270, 1160; EIMS *m/z* (rel. int.): 316 $[M]^+$ (100), 301 (40), 283 (12), 265 (8), 210 (90), 109 (81); ^1H NMR: Table 2; ^{13}C NMR: Table 3.

3.3.10. 3-(3,4-Dimethoxybenzyl)-5,7-dihydroxychroman-4-one (**10**)

White powder, m.p. 183–185°C; $[\alpha]_D^{25}$ −74.7 (MeOH, *c* 0.39); UV λ_{max}^{MeOH} nm (log ε): 214 (4.38), 288 (4.35); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 223 (4.40), 312 (4.43) 378 (3.55): $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 222 (4.41), 310 (4.42), 378 (3.58); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 226 (4.18), 325 (4.49); IR ν_{max}^{KBr} cm^{-1}: 3410 (–OH), 1650 (C=O), 1615, 1515, 1385, 1260, 1160, 1075, 1025, 840; EIMS *m/z* (rel. int.): 330 $[M^+]$ (98), 316 (10), 179 (15), 178

(70), 152 (30), 151 (100), 124 (16), 107 (15); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3

*3.3.11. 3-(4-Methoxybenzyl)-6-hydroxy-5,7-dimethoxychroman-4-one (**11**)*

Yellow oil; $[\alpha]_D^{25}$ −68.7 (MeOH, *c* 0.26); UV λ_{max}^{MeOH} nm (log ε): 210 (4.32), 281 (4.10), 344 (3.63); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 208 (4.31), 281 (4.10), 344 (3.69); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): no change; $\lambda_{max}^{MeOH+MeONa}$ nm (log ε): no change; IR ν_{max}^{KBr} cm^{-1}: 3415 (–OH), 2930, 1665 (C=O), 1610, 1500, 1450, 1250, 1100, 1030, 820; EIMS *m/z* (rel. int.): 344 [M$^+$] (100), 316 [M$^+$-CO] (15), 224 (10), 223 (20), 197 (15), 196 (25), 122 (15), 121 (94); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

*3.3.12. 3-(4-Hydroxybenzyl)-5,6,7-trimethoxychroman-4-one (**12**)*

Yellow oil; $[\alpha]_D^{25}$ −231.9 (MeOH, *c* 0.13); UV λ_{max}^{MeOH} nm (log ε): 223 (4.42), 276 (4.22), 322 (3.67); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 224 (4.40), 276 (4.21), 319 (3.70); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 227 (4.58), 276 (4.27), 323 (3.86); IR ν_{max}^{KBr} cm^{-1}: 3450 (–OH), 2925, 2840, 2340, 1655 (C=O), 1610, 1450, 1390, 1260, 1110; EIMS *m/z* (rel. int): 344 [M$^+$] (100), 329 [M$^+$-CH$_3$] (25), 316 [M$^+$-CO] (60), 237 (40), 223 (16), 210 (42), 195 (76), 167 (41) 137 (38), 107 (45); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

*3.3.13. 3-(4-Methoxybenzyl)-8-hydroxy-5,7-dimethoxychroman-4-one (**13**)*

Yellow oil; $[\alpha]_D^{25}$ −109.9 (MeOH, *c* 0.23); UV λ_{max}^{MeOH} nm (log ε): 205 (4.39), 285 (4.13), 345 (3,60); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): no change; IR ν_{max}^{KBr} cm^{-1}: 3440 (–OH), 2940, 1660 (C=O), 1590, 1270, 1100, 830; EIMS *m/z* (rel. int.): 344 [M$^+$] (100), 329 [M$^+$-CH$_3$] (10), 313 (15), 237 (15), 223 (24), 197 (20), 196 (36), 181 (38), 147 (56), 121 (96); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

*3.3.14. 3′,4-Dihydroxy-3,5′-dimethoxystilbene (**14**)*

Brown oil; IR ν_{max}^{KBr} cm^{-1}: 3450 (–OH), 2930, 2360, 1610, 1510, 1450; ^{1}H NMR (CD_3COCD_3) δ: 3.77 (3H, s, OMe), 3.89 (3H, s, OMe), 6.31 (1H, t, *J* = 1.8 Hz, H-4′), 6.62 (1H, br d, *J* = 1.8 Hz, H-2′), 6.63 (1H, br d, *J* = 1.8 Hz, H-6′), 6.82 (1H, d, *J* = 8.2 Hz, H-5), 6.97 (1H, d, *J* = 16.2 Hz, H-α), 7.02 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 7.10 (1H, d, *J* = 16.2 Hz, H-β), 7.22 (1H, d, *J* = 1.8 Hz, H-2); ^{13}C NMR (CD_3COCD_3): 161.5 (C-3′), 159.1 (C-5′), 148.0 (C-4), 147.1 (C-3), 140.3 (C-1′), 129.7 (C-1), 129.2 (C-α), 126.3 (C-β), 120.7 (C-6), 115.4 (C-5), 109.5 (C-2), 106.1 (C-2′), 103.2 (C-6′), 100.8 (C-4′); EIMS *m/z* (rel. int.): 272 [M$^+$] (100).

*3.3.15. 3,3′,5′-Trihydroxy-4-methoxystilbene, Rhapontigenin (**15**)*

Brown oil; ^{1}H NMR (CD_3COCD_3) δ: 3.88 (3H, s, OMe), 6.35 (1H, br t, *J* = 1.8 Hz, H-4′), 6.61 (2H, br d, *J* = 1.8 Hz, H-2′ and H-6′), 6.85 (1H, d, *J* = 8.3 Hz, H-5), 6.94 (1H, d, *J* = 16.2 Hz, H-α), 7.02 (1H, dd, *J* = 8.3, 1.8 Hz, H-6), 7.04 (1H, d, *J* = 16.2 Hz, H-β), 7.20 (1H, d, *J* = 1.8 Hz, H-2); ^{13}C NMR (CD_3COCD_3) δ: 159.4 (C-3′, C-5′), 148.5 (C-3), 147.4 (C-4), 140.8 (C-1′), 130.4 (C-1), 129.4 (C-α), 127.0 (C-β), 121.1 (C-6), 116.0 (C-5), 110.1 (C-2), 105.8 (C-2′ and C-6′), 102.8 (C-4′), 56.2 (OMe); EIMS *m/z* (rel. int.): 258 [M$^+$] (100).

*3.3.16. 4,3′,5′-Trihydroxy-3-methoxystilbene, Isorhapontigenin (**16**)*

Brown oil; ^{1}H NMR (CD_3COCD_3) δ: 3.80 (3H, s, OMe), 6.35 (1H, br t, *J* = 1.8 Hz, H-4′), 6.61 (2H, br d, *J* = 1.8 Hz, H-2′, H-6′), 6.90 (1H, d, *J* = 8.1 Hz, H-5), 6.92 (1H, d, *J* = 16.4 Hz, H-α), 6.97 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 7.00 (1H, d, *J* = 16.4 Hz, H-β), 7.12 (1H, d, *J* = 2.0 Hz, H-2); ^{13}C NMR (CD_3COCD_3) δ: 159.4 (C-3′, C-5′), 148.3 (C-4), 147.3 (C-3), 140.6 (C-1′), 130.4 (C-1), 129.1 (C-α), 127.5 (C-β), 119.9 (C-6), 113.3 (C-5), 112.4 (C-2), 105.7 (C-2′, C-6′), 102.8 (C-4′), 56.2 (OMe); EIMS *m/z* (rel. int.): 258 [M$^+$] (100).

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