PERGAMON

Phytochemistry 51 (1999) 947–951

PHYTOCHEMISTRY

Homoisoflavanones and stilbenoids from *Scilla nervosa*

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Received 10 December 1998; received in revised form 3 March 1999; accepted 3 March 1999

Abstract

The bulbs of *Scilla nervosa* have yielded three novel compounds, 5,7-dimethoxy-3-(4-methoxybenzyl)chroman-4-one, 5-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)-chroman-4-one, 5,7-dimethoxy-3-(4-hydroxybenzyl)chroman-4-one and the known compounds 5-hydroxy-7-methoxy-3-(4-methoxybenzyl)chroman-4-one, (*E*)-5,7-dihydroxy-3-(4-hydroxybenzylidene) chroman-4-one, (*E*)-resveratrol and (*E*)-3,3′,5-trihydroxy-4′-methoxystilbene (rhapontigenin).

Keywords: *Scilla nervosa*; Hyacinthaceae; Homoisoflavanones; (*E*)-resveratrol; (*E*)-3; 3′; 5-trihydroxy-4′-methoxystilbene

1. Introduction

Scilla nervosa (Burch.) Jessop (syn. *Schizocarphus rigidifolia* Kunth)(Hyacinthaceae) is the most widespread of the southern African representatives of the genus *Scilla* L. It has long been recognised as a poisonous species (Bryant, 1909) particularly to stock (Kellerman, Coetzer & Naude, 1988; Watt & Breyer-Brandwijk, 1962) but nonetheless holds an important place in local pharmacopoeias. Previous studies on *Scilla* species have shown them to contain triterpenoid (Mimaki et al., 1993) and cardiac (Kamano & Petit, 1974) glycosides as well as homoisoflavanones (Kuono, Komori & Kawasaki, 1973; Heller & Tamm, 1981). Traditional use of *S. nervosa* has been widely documented: bulbs have been used to treat nervous conditions in children and as a treatment for dysentery (Watt & Breyer-Brandwijk, 1962), and diluted bulb decoctions as analgesics in the treatment of rheumatic fever (Bryant, 1909). Bulbs are used by the Sotho to treat gall sickness in livestock and crushed bulbs are added to food and administered to children as an aperient (Hedberg & Staugard, 1989). The Tswana use the cooked bulbs mixed with porridge and eaten three to four times a day for a month for the treatment of infertility in women (Jacot Guillarmod, 1971). Two samples of *S. nervosa* were obtained. The first specimen (KZN) was purchased from the Warwick Triangle herbal market in Durban. A second collection (MPL) was made in Lydenburg, Mpumalanga. Five homoisoflavanones, three of which have not been described previously, and two known stilbenoids were isolated. Structural elucidation was performed using NMR, UV (including use of shift reagents) and MS techniques. The known anti-histaminic (Amschler et al., 1996) and anti-inflammatory properties (Della Logia, Del Negro, Tubaro, Barone & Parilli, 1989) of homoisoflavanones would account for the use of *S. nervosa* by the Zulu in the treatment of rheumatic fever (Bryant, 1909).

2. Results and discussion

S. nervosa (KZN) yielded compounds **1**, **2**, **3**, **5**, **6** and **7** and the MPL sample again yielded compound **1** and also compound **4.** Compounds **1**, **2** and **3** have not been reported previously but **4**, **5**, **6** and **7** are

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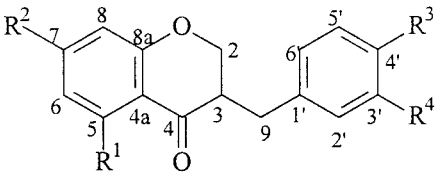
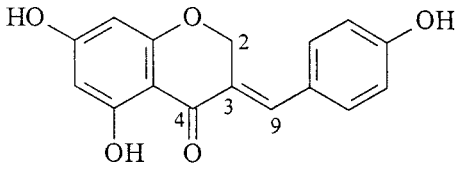
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00155-7



	R^1	R^2	R^3	R^4
1	OCH_3	OCH_3	OCH_3	H
2	OH	OCH_3	OCH_3	OH
3Ac	OCH_3	OCH_3	$OCOCH_3$	H
4	OH	OCH_3	OCH_3	H



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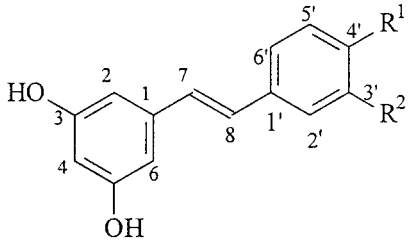

	R^1	R^2
6	OH	H
7	OCH_3	OH

known and their structures were confirmed by comparison of NMR and other data against literature values (Heller, Andermatt, Schaad & Tamm, 1976; Finckh & Tamm, 1970; Adinolfi, Lanzetta, Laonigro, Parrilli & Breitmaier, 1986; Jayatilake et al., 1993; Hanawa, Takara & Mizutani, 1992; Kashiwada, Nonaka & Nishioka, 1984; Adinolfi, Aquilla, Barone, Lanzetta & Parrilli, 1989; Masterova et al., 1991).

The COSY spectrum of **1** ($C_{19}H_{20}O_5$) indicated the compound was a homoisoflavanone with resonances ascribable to the two protons at C-2 occurring at δ4.11 and δ4.29, the proton at C-3 occurring as a multiplet at δ2.72 and the two protons at C-9 each occurring as double doublets at δ2.68 and δ3.10. Similar resonances were present in **2**, **3** and **4**, indicating these compounds were also homoisoflavanones. Two singlets, the first integrating to three protons and the second to six protons, occurred at δ3.80 and δ3.86 respectively indicating three methoxy groups. A pair of doublets, each integrating to two protons, at δ7.17 and δ6.89 (J 8.7 Hz) indicated a *para*-disubstituted ring B. Resonances ascribable to H-6 and H-8 (δ6.15, δ6.20, J 2.4 Hz) indicated substitution at C-5 and C-7. Thus the methoxy groups were placed at C-5, C-7 and C-4′. A keto group at C-4 was indicated by a resonance at δ193.9 in the ^{13}C NMR spectrum. The mass spectrum confirmed the structure with ions at m/z 180 and 181 due to *retro* Diels–Alder cleavage and hydrogen shift of a chromanone fragment and at m/z 207 as a result of an A-4 type cleavage of the molecular ion (Heller & Tamm, 1981). Thus compound **1** is 5,7-dimethoxy-3-(4-methoxybenzyl)chroman-4-one.

The ^{1}H NMR spectrum of **2**, $C_{18}H_{18}O_6$, showed resonances ascribable to protons of two methoxy groups at δ3.84 and δ3.86. The molecular formula indicated the presence of two hydroxy groups. Resonances ascribable to H-6 and H-8 occurred as doublets at δ6.03 and δ6.07 (J 2.4 Hz) indicating substitution at C-5 and C-7. The mass spectrum showed peaks at m/z 193, 166 and 167 corresponding to A-4, reverse Diels–Alder and H-shift fragments respectively indicating the presence of a methoxy and hydroxy substituent on

ring A. A positive and negative bathochromic shift in the UV spectrum of **2** with $AlCl_3$ and NaOAc respectively indicated a 5-hydroxy-7-methoxy-substituted ring A (Heller & Tamm, 1981). An ABX system in the ^1H NMR spectrum indicated that ring B was trisubstituted. The base peak at *m/z* 137 corresponded to a hydroxy-methoxybenzyl/tropylium ion indicating the presence of a hydroxy and a methoxy substituent on ring B. The position of these groups on ring B was determined by NOE experiments. Irradiation of a double doublet at δ6.71 ($J=2.1$, 8.1) ascribable to H-6′ led to the enhancement of a doublet due to H-5′ at δ6.89 ($J=8.1$ Hz). Irradiation of this signal led to enhancement of the methoxy group proton singlet at δ3.86. A doublet at δ6.75 showed *meta*-coupling ($J=2.1$ Hz) with H-6′, hence it was assigned to H-2′. The remaining hydroxy group was therefore placed at C-3′. Thus **2** is 5-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one.

Compound **3** proved difficult to purify and a mixture containing **3** was acetylated in order to obtain a pure acetylated derivative, **3Ac**. The molecular formula of **3Ac** was found to be $C_{20}H_{20}O_6$. Ring A possessed 5,7-dimethoxy substituents as in **1** and ^1H NMR spectroscopy showed a *para*-disubstituted ring B as in **1**. However doublets ascribable to H-2′/H-6′ and H-3′/H-5′ had been shifted downfield compared to their positions in **1** due to the presence of the acetyl group at C-4′. Thus the original compound **3** would have been 5,7-dimethoxy-3-(4-hydroxybenzyl)chroman-4-one.

Compound **4** was found to be 5-hydroxy-7-methoxy-3-(4-methoxybenzyl)chroman-4-one, previously reported from *Eucomis bicolor* (Hyacinthaceae) (Heller et al., 1976). The optical rotations of compounds **1**, **2** and **4** were found to be −50, −36, and −38° respectively. This differs from values for synthetic samples of **1** and **4** which were reported as +12 and +23° respectively (Heller et al., 1976). The configuration at C-3 could not be determined. The structure of compound **5** was determined to be 5,7-dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one. This compound differed from the other homoisoflavanones in having a 3(9)-double bond. There are contradictions in the literature regarding the configuration of the C-3, C-9 double bond for this compound (Heller & Tamm, 1981; Heller et al., 1976; Adinolfi et al., 1989; Masterova et al., 1991), hence NOE experiments were performed. Irradiation of the H-9 resonance gave an enhanced signal for the H-2′/H-6′ doublet but not the H-2 signal. Irradiation of the H-2 signal led to the enhancement of the H-2′/H-6′ doublet, but no effect was observed for the H-9 signal. Thus the configuration of the C-3,C-9 double bond was deduced to be (*E*). This compound has been reported before from *Eucomis comosa* (syn. *E. punctata*) (Hyacinthaceae) (Finckh & Tamm, 1970).

Compounds **6** and **7** were found to be (*E*)-3,4′,5-trihydroxystilbene ((*E*)-resveratrol) and (*E*)-3,3′,5-trihydroxy-4′-methoxystilbene (rhapontigenin) which were first isolated from *Veratrum grandiflorum* (Melanthiaceae) and *Rheum rhizoma* (Polygonaceae) respectively (Jayatilake et al., 1993; Hanawa et al., 1992; Kashiwada et al., 1984). The presence of stilbenoids has not been reported previously in the Hyacinthaceae.

3. Experimental

3.1. General

Fresh bulbs of *S. nervosa* (Burch.) Jessop (1.2 kg) were purchased at the Warwick Triangle Herbal Market in Durban and a voucher specimen retained in the Natal Herbarium (*Bangani and Crouch 2*, NH). The bulbs were cut into small pieces, air dried overnight, and extracted successively using a soxhlet apparatus with hexane, chloroform, ethyl acetate and methanol for 24 h with each solvent. The hexane and chloroform extracts both yielded **1** and **2.** The ethyl acetate extract yielded impure **3** and **4**, **5**, **6** and **7** after CC on silica gel (Merck, 9385). Compound **3** was isolated as its acetate, **3Ac**, after treatment in the usual way with Ac_2O/py. Compounds **4**, **5**, **6** and **7** were identified as the previously reported 5-hydroxy-7-methoxy-3-(4-hydroxybenzyl)chroman-4-one, 5,7-dimethoxy-3-(4-hydroxy benzylidene)chroman-4-one (Amschler et al., 1996; Della Logia et al., 1989), (*E*)-3,4′,5-trihydroxystilbene, (*E*)-3,3′,5-trihydroxy-4′-methoxystilbene (Jayatilake et al., 1993; Hanawa et al., 1992; Kashiwada et al., 1984).

A second sample (950 g) was collected in Lydenburg, Mpumalanga (*Crouch 752*, NH). Bulbs were cut into pieces, dried overnight, and shaken in methanol (2.5 L) for 24 h. After removal of methanol, water was added and the crude extract extracted with hexane, ether and ethyl acetate. The hexane extract again yielded **1** and **4**.

NMR spectra were recorded in CD_3OD on a Varian 300 MHz spectrometer. ^1H and ^{13}C NMR data of **1**–**5** are given in Tables 1 and 2.

3.2. 5,7-dimethoxy-3-(4-methoxybenzyl)chroman-4-one (*1*) (30 mg KZN, 45 mg MPL), vitreous solid

HRMS: M^+ at *m/z* 328.1306 ($C_{19}H_{20}O_5$ requires 328.1311), EIMS: 328(100), 207(37), 181(35), 148(26), 121(90). IR ν_{max}(NaCl)cm^{-1}:2932, 1686, 1608, 1524, 1259, 1041. UV λ_{max} nm(log ϵ):283(4.47), no batho-

Table 1
^{1}H NMR data of compounds **1**, **2**, **3Ac**, **4**, **5** (300 MHz, CD_3 OD)

Cpd	2H-2	H-3	H-6	H-8	2H-9	H-2′	H-6′	H-3′	H-5′	Other signals
1	4.11, 4.29	2.72 *m*	6.20 *d*	6.15 *d*	2.68*dd* (9.8, 12.6)	7.17 *d*		6.89 *d*		3.80 *s* 5-OCH_3
	AB of ABX (3.8, 6.6, 11.4)		AB (2.4)		3.10*dd* (3.6, 12.6)	AA′BB′ (8.7)		AA′BB′ (8.7)		3.86 *s* 7-OCH_3
										3.86 *s* 4′-OCH_3
2	4.14, 4.31	2.87 *m*	6.07 *d*	6.03 *d*	2.67*dd* (10.2, 13.8)	6.75 *d* (2.1)	6.71*dd* (2.1; 8.1)	–	6.89 *d* (8.1)	3.84 *s* 7-OCH_3
	AB of ABX (4.2, 7.2, 11.4)		AB (2.4)		3.10*dd* (3.6, 13.8)					3.86 *s* 4′-OCH_3
3Ac	4.15, 4.32	2.82 *m*	6.23 *d*	6.18 *d*	2.72 *m*	7.31 *d*		7.07 *d*		3.86 *s* OCH_3
	AB of ABX (4.2, 7.2, 11.4)		AB (2.4)		3.16 *m*	AA′BB′ (8.4)		AA′BB′ (8.4)		3.86 *s* OCH_3
										2.30 *s* 4′-OAc
4	4.13, 4.31	2.88 *m*	6.07 *d*	6.03 *d*	2.72 *d* (9.9, 13.5)	7.19 *d*		6.90 *d*		3.84 *s* 7-OCH_3
	AB of ABX (4.2, 7.2, 11.4)		AB (2.1)		3.17*dd* (4.5, 13.8)	AA′BB′ (8.7)		AA′BB′ (8.7)		3.81 *s* 4′-OCH_3
5	5.35 *d* (1.8)	–	5.93 *d*	5.87 *d*	7.77*t* (1.8)	7.30 *d*		6.92 *d*		–
			AB (2.2)			AA′BB′ (8.7)		AA′BB′ (8.4)		

chromic shifts on addition of $AlCl_3$ or NaOAc. $[\alpha]_D = -50^\circ$ (*c* 0.3 g/100 ml, CH_3OH).

*3.3. 5-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one (**2**) (62 mg KZN), pale yellow crystals, mp 115–117°C*

HRMS: M^+ at *m/z* 330.1092 ($C_{18}H_{18}O_6$ requires 330.1093). EIMS: 330(37), 193(7),167(21), 166(3), 164(4), 137(100). IR ν_{max}(NaCl)cm^{-1}:3461, 2953, 2835, 1645, 1581, 1515, 1279, 1168. UV λ_{max} nm (log ϵ):288(4.34). + $AlCl_3$ 312, no shift on addition of NaOAc. $[\alpha]_D = -36^\circ$ (*c* 0.55 g/100 ml, CH_3OH).

Table 2
^{13}C NMR data of compounds **1**, **2**, **3Ac**, **4**, **5**, **6**, **7** (75 MHz, CD_3 OD)

C	**1**	**2**	**3Ac**	**4**	**5**	**6**	**7**
1	–	–	–	–	–	141.3	141.1
2	70.1	70.4	70.1	70.4	68.6	105.8	105.9
3	[a]	[a]	[a]	[a]	127.0	159.7	159.7
4	193.9	199.8	193.7	199.8	186.5	102.7	102.8
4a	106.1	103.6	106.1	103.8	103.8	–	–
5	164.0	165.6	164.1	165.6	164.0	159.7	159.7
6	93.8	95.9	93.9	95.8	97.4	105.8	105.9
7	168.0	169.4	168.1	170.0	169.4	129.4	129.4
8	94.6	94.6	94.6	94.6	96.0	127.0	127.9
8a	166.6	164.6	166.7	164.6	160.7	–	–
9	33.3	33.1	33.4	32.9	138.0	–	–
1′	131.8	132.3	130.5	131.4	128.4	130.4	132.2
2′	131.1	117.1	131.2	131.2	133.6	128.8	113.6
3′	115.0	147.9	122.9	115.1	116.8	116.5	149.0[b]
4′	159.9	147.7	137.7	160.0	160.7	158.4	147.7[b]
5′	115.0	113.0	122.9	115.1	116.8	116.5	112.7
6′	131.1	121.4	131.2	131.2	133.6	128.8	120.0
5-OCH_3	56.3	–	56.3	–	–	–	–
6-OCH_3	–	–	–	–	–	–	–
7-OCH_3	56.2	56.5	56.3	55.7	–	–	–
4′-OCH_3	55.7	56.3		56.3	–	–	56.4
4′-OCO$\underline{CH_3}$	–	–	20.9	–	–	–	–
4′-O$\underline{CO}CH_3$	–	–	171.1				

[a] Hidden under solvent peaks.
[b] Values can be interchanged.

*3.4. 5,7-dimethoxy-3-(4-hydroxybenzyl)chroman-4-one (**3**)*

Acetylation of an impure sample of **3** yielded, after CC, **3Ac,** *5,7-dimethoxy-3-(4-acetoxybenzyl)chroman-4-one* (12 mg),vitreous off-white solid. HRMS: M^+ at *m/z* 356.1270($C_{20}H_{20}O_6$ requires 356.1260). EIMS: 356(75), 314(41), 207(67), 181(100), 180(63), 107(19). IR ν_{max}(NaCl)cm^{-1}:2930, 2857, 1729, 1671, 1613, 1466, 1228, 1165. UV λ_{max} nm(log ϵ):282(4.17), no bathochromic shifts on addition of $AlCl_3$ or NaOAc. $[\alpha]_D$ not measured (sample too dilute).

Acknowledgements

This research was funded by the University of Natal Research Fund, the Foundation for Research Development. NC gratefully acknowledges assistance from the Mazda Wildlife Fund, and the DEAT in their sponsorship of MEDBASE. We thank the Natal Parks Board and the Mpumalanga Parks Boards for permits to collect plant materials and Dr P Boshoff for running mass spectra. VB is grateful for bursaries from the Foundation for Research Development and the Mellon Foundation.

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