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Homoisoflavonoids and xanthones from the tubers of wild and in vitro regenerated *Ledebouria graminifolia* and cytotoxic activities of some of the homoisoflavonoids

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Abstract

Eleven homoisoflavonoids and two xanthones were isolated and characterized from the bulbs of *Ledebouria graminifolia*. Five of the homoisoflavonoids are new compounds and were identified as: 5-hydroxy-7-methoxy-3-(4′-hydroxybenzyl)-4-chromanone, 5-hydroxy-6,7-dimethoxy-3-(4′-hydroxybenzyl)-4-chromanone, 5,7,8-trimethoxy-3-(4′-hydroxybenzyl)-4-chromanone, 5-hydroxy-3′,4′,7-trimethoxyspiro{2H-1-benzopyran-7′-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one, 5,7-dihydroxy-3′,4′-dimethoxyspiro{2H-1-benzopyran-7′-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one. Structures were elucidated by extensive 1D, and 2D NMR spectroscopy and HRMS. A method for tissue culture was developed and the bulbs of mature plants were found to contain all the compounds isolated from the wild specimens of *L. graminifolia*.

Keywords: *Ledebouria graminifolia*; Hyacinthaceae; *Maloba*; Bulb; Structure elucidation; Tissue culture; Anti-tumor activity; Spectroscopy; Homoisoflavonoids; Xanthones

1. Introduction

Samples of a widely traded bulb collected from the wild is sold in stalls at the Gaborone Railway Station (Botswana) under the name of 'maloba'. The scientific name could not be determined and so it was grown in a pot to yield a botanical specimen which was identified as *Ledebouria graminifolia* (Bak.) Jessop (Hyacinthaceae). The chemistry of this family has been reviewed recently (Crouch et al., 1999a). *L. graminifolia* is one of six species belonging to the genus *Ledebouria* recorded in Botswana. They are widely used in traditional medicine, primarily for skin irritations, for wound dressing, sores, coughs, backaches, gastroenteritis and as a soothing medicine during pregnancy (Hutchings, 1996; Watt and Breyer-Brandwijk, 1962). *L. graminifolia* is believed to have attributes for cleansing urinary tracts and treatment of heart disease. To our knowledge, no scientific work has so far been reported on this plant although it has become a traded commodity for its medicinal properties. In vitro regeneration procedures led to successful micropropagation of the plant. Mature *L. graminifolia* plants obtained from tissue culture produced bulbs which are morphologically similar to the wild ones. The chemical constituents of the cultured bulbs were compared with those of the bulbs purchased from the market.

2. Results and discussion

Flash chromatography of the organic extract of the bulbs of *L. graminifolia*, followed by separations employing Sephadex LH-20 and PTLC yielded seven 3-benzyl-4-chromanones (**1**–**7**), two 3-benzyl-3-hydroxy-4-chromanones (**8** and **9**), two scillascillin type homoisoflavonoids (**10** and **11**) and two xanthones (**12**–**13**).

2.1. 3-Benzyl-4-chromanones (*1*–*7*)

The ^{1}H NMR spectrum of each of the 3-benzyl-4-chromanones (**1**–**7**) was characterized by the presence of two sets of an ABX system attributable to the presence

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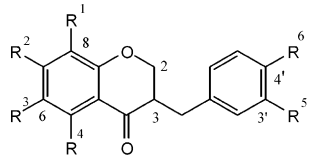
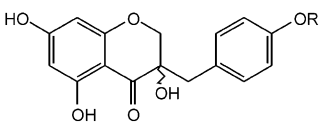
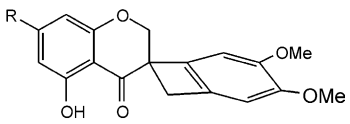


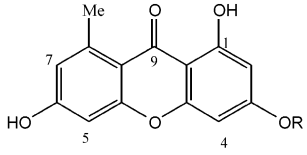
	R^1	R^2	R^3	R^4	R^5	R^6
1	H	HO	H	HO	H	HO
2	H	HO	MeO	HO	H	HO
3	H	MeO	H	HO	H	HO
4	H	HO	H	HO	H	MeO
5	H	MeO	MeO	HO	H	HO
6	MeO	MeO	H	OMe	H	HO
7	H	HO	H	HO	HO	HO



8 R = H
9 R = Me



10 R = OH
11 R = OMe



12 R = H
13 R = Me

of non-equivalent protons at C-2 and C-9. These signals were observed at δ 4.0–4.3 and 2.5–3.0 ppm, respectively. The methine proton at C-3 gave resonance signals at about δ 2.8 (Table 1). The ^{13}C NMR spectra also exhibited signals for C-2, C-3 and C-9 carbons at δ_C 69, 48 and 32, respectively. These spectral characteristics are typical for 3-benzyl-4-chromanones found in *Scilla nervosa* subsp. *rigidifolia* by ourselves (Silayo et al., 1999) and from *Ledebouria* species by others (Pohl et al., 2000). The ^{1}H NMR spectra of **1**–**6** showed an

Table 1
^{1}H NMR spectral data, δ (*J* Hz) for compounds **1**, **2**, **4**, **5**, **7** in CD_3COCD_3 and **3**, **6** in CD_3OD (300 MHz)

	1	**2**	**3**	**4**	**5**	**6**	**7**
H-2a	4.30, *dd*, (4.5, 11.4)	4.43, *dd*, (4.5, 11.5)	4.22, *dd*, (3.6, 11.3)	4.30, *dd*, (4.5, 11.3)	4.29, *dd*, (4.4, 11.3)	4.34, *dd*, (3.7, 11.3)	4.28, *dd*, (4.5, 11.3)
H-2b	4.12, *dd*, (8.0, 11.4)	4.24, *dd*, (7.9, 11.3)	4.05, *dd*, (6.4, 11.3)	4.11, *dd*, (8.1, 11.5)	4.09, *dd*, (8.3, 11.1)	4.18 ,*dd*, (6.5, 11.3)	4.10, *dd*, (8.0, 11.4)
H-3	2.88, *m*	2.93, *m*	2.67, *m*	2.92, *m*	2.75, *m*	2.74, *m*	2.85, *m*
H-6	5.91	–	5.96, *d*, (2.2)	5.91	–	6.34	5.88, *d*, (2.1)
H-8	5.93	5.99	6.08, *d*, (2.0)	5.93	6.36	–	5.91, *d*, (2.1)
H-9a	3.14, *dd*, (4.5, 13.8)	3.15, *dd*, (4.6, 13.8)	3.03, *dd*, (3.8, 12.7)	3.17, *dd*, (4.7, 14.0)	3.11, *dd*, (4.3, 13.6)	3.06, *dd*, (4.1, 13.1)	3.08, *dd*, (4.4, 13.8)
H-9b	2.67, *dd*, (10.0, 13.8)	2.72, *dd*, (9.8, 13.8)	2.60, *dd*, (8.7, 12.7)	2.72, *dd*, (10.0, 13.9)	2.61, *dd*, (10.0, 13.4)	2.65, *dd*, (10.1, 12.9)	2.59, *dd*, (10.2, 13.8)
H-2′	7.12, *d*, (8.3)	7.13, *d*, (8.3)	7.05, *d*, (8.5)	7.22, *d*, (8.6)	7.10, *d*, (8.5)	7.07, *d*, (8.5)	6.59, *dd*, (2.0, 8.8)
H-3′	6.81, *d*, (8.4)	6.81, *d*, (8.5)	6.74, *d*, (8.5)	6.90, *d*, (8.6)	6.80, *d*, (8.5)	6.74, *d*, (8.5)	6.77, *d*, (8.0)
H-5′	7.12, *d*, (8.3)	6.81, *d*, (8.5)	6.74, d, (8.5)	6.90, *d*, (8.6)	6.80, *d*, (8.5)	6.74, d, (8.5)	–
H-6′	6.81, *d*, (8.4)	7.13, *d*, (8.3)	7.05, *d*, (8.5)	7.22, *d*, (8.6)	7.10, *d*, (8.5)	7.07, d, (8.5)	6.77, *d*, (2.0)
5-OH	12.26	12.03	–	12.25	–	–	12.20
5-OMe	–	–	–	–	–	3.90	–
6-OMe	–	3.73	–	–	3.81	–	–
7-OMe	–	–	3.82	–	3.90	3.96	–
8-OMe	–	–	–	–	–	3.73	–
4′-OMe	–	–	3.79	–	–	–	

AA′BB′ system due to the protons of ring B and the assignment was further confirmed by the ^{13}C NMR spectra (δ_C 130 for C-2′/6′ and δ_C 115 for C-3′/5′) (Table 2). The ^1H NMR spectrum of compound **7** differed from those of **1**–**6** in having an ABX system indicating that ring B was substituted differently. The spectra of compounds **1**, **3**, **4** and **7** further showed the presence of *meta* coupled signals and these were assigned to H-6 and H-8 of ring A. The chemical shifts of these protons were observed between δ 5.88–5.96 (H-6) and 5.91–6.08 (H-8), indicating the C-5 and C-7 positions were oxygenated. The forgoing analysis together with the EIMS data [*m*/z 286 (100) $[M]^+$ for **1**, and *m*/*z* 302 (23) $[M]^+$ for **7**] led to the assignment of the given structures for compounds **1** and **7**. Compound **7** is known to occur in *Muscari comosum* and *S. nervosa* spp *rigidifolia* (Adinolfi et al., 1985; Silayo et al., 1999) while **1** was found in *M. comosum*. The spectroscopic data given for these compounds in these reports were identical to those measured by us in this study. The spectra of **3** and **4**, each showed the presence of one methoxy group, the location of which was deduced from the MS fragmentation pattern. In the case of **3** an ion formed by the cleavage of the C3–C9 bond was noted at *m*/*z* 107, while **4** showed a prominent ion at *m*/*z* 121 instead (Heller and Tamm, 1981). This is consistent with the presence of the methoxy group at 4′ in the latter and at the C-5 or C-7 position in the former. The data obtained for **4** was found to be identical with those of 5,7-dihydroxy-3-(4′-methoxybenzyl)-4-chromanone isolated from *Eucomis bicolor* by Heller et al (1976). The position of the methoxy group in **3** was assigned to be at C-7 based on UV shift reagent data (Experimental) and the observed NOE enhancement of the H-6 and H-8 upon irradiation of the methoxy signal. HREIMS data, 300.1012 (calc. 300.09997) was consistent with the proposed molecular formula $C_{17}H_{16}O_5$. Compounds **2**, **5** and **6**, were found to contain one, two and three methoxy groups, respectively, and that these groups were located in ring A was deduced from MS data, since in each case, a prominent ion was observed at *m*/*z* 107 (and not at 121, which would have been the case for methoxylated ring B). The ^1H NMR of **2** and **5** each indicated a sharp singlet at δ 5.99 and 6.36, respectively, and these were assigned to H-8 in each compound. HMBC correlations were used to confirm the assignments by observing ^3J correlations of each of these signals to C-8a, which in turn showed correlation to the unambiguously assigned proton signals of H-2. The location of the only methoxy signal observed in **2** was deduced to be at C-6 on the basis of the downfield resonance position of the methoxy carbon at δ_C 60.7 and the presence of a chelated –OH signal, which requires the presence of a C-5 OH group. Compound **2** was recently reported from *S. nervosa* from our own laboratories and a direct comparison of the two samples was possible for confirmation of identity (Silayo et al., 1999). The location of two methoxy groups of **5** at C-6 and C-7 were confirmed by similar spectroscopic (NMR and UV shift data) data. The structure of **5** was further supported by HREIMS data: 330.1103 (calc. for $C_{18}H_{18}O_6 = 330.1103$).

The ^1H NMR of **6** showed a sharp singlet resonating at δ 6.34 and three methoxy signals. Unlike in the spectra of compounds **2** and **5**, the proton signal at δ 6.34

Table 2
^{13}C NMR spectral data for compounds **1**, **2**, **4**, **5**, **7**, **8**, **9**, **11** in CD_3COCD_3 and **3**, **6**, **10** CD_3OD (300 MHz)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
2	69.3	69.8	68.8	70.2	69.6	70.1	69.3	70.2	72.0	73.7	73.9
3	46.2	46.8	48.9	46.7	48.8	49.0	–	71.7	72.3	54.4	54.5
4	197.9	198.6	193.0	198.7	190.8	194.0	–	198.7	198.1	196.1	194.1
4a	101.7	102.3	104.2	102.6	108.8	105.1	–	102.5	99.7	100.7	102.4
5	164.8	154.7	165.9	165.7	147.1	160.4	–	165.1	164.8	162.8	166.5
6	95.4	129.2	95.8	96.2	135.1	90.5	98.8	96.3	96.0	95.3	95.1
7	168.2	160.3	163.5	169.0	154.5	159.6	–	169.0	163.4	164.9	168.4
8	95.4	96.1	93.3	96.2	96.1	130.2	95.5	95.5	96.0	92.5	94.1
8a	163.7	159.7	165.5	164.6	156.9	156.9	–	164.6	163.4	163.9	163.8
9	31.8	31.8	32.4	32.6	32.0	33.1	–	32.7	40.1	35.3	35.2
1′	129.3	128.7	129.6	–	129.9	–	120.6	131.3	127.5	134.6	134.3
2′	130.4	130.5	130.1	131.3	130.4	131.0	130.0	130.2	132.1	108.5	108.8
3′	115.7	115.7	115.3	115.2	115.6	116.2	115.7	116.5	113.7	151.5	150.8
4′	156.4	156.6	156.1	159.8	156.4	156.9	144.2	157.3	159.2	150.3	152.1
5′	115.7	115.7	115.3	115.2	115.6	116.2	145.5	116.5	113.7	106.7	107.1
6′	130.4	130.5	130.1	–	130.4	131.0	–	130.2	132.1	136.3	136.0
5-OMe	–	–	–	–	–	56.2	–	–	–	–	–
6-OMe	–	60.7	–	–	60.7	–	–	–	–	–	–
7-OMe	–	–	55.2	–	56.0	56.5	–	–	–	–	55.8
8-OMe	–	–	–	–	–	61.2	–	–	–	–	–
3′-OMe	–	–	–	–	–	–	–	–	–	55.9	56.1
4′-OMe	–	–	–	55.8	–	–	–	–	54.9	55.9	56.0

showed no long-range correlation to C-8a, hence it was assigned to H-6. The ^{1}H NMR spectrum of 5,7,8-trimethoxy-3-(4′-hydroxybenzyl)-4-chromanone (**6**) was similar to that of 5,6,7-trimethoxy-3-(4′-hydroxybenzyl)-4-chromanone isolated from *S. nervosa* by Silayo et al. (1999), however, the two compounds exhibit different R_f values (0.45 and 0.50, respectively, in $CHCl_3$/MeOH 4.8:0.2; 0.24 and 0.43, respectively, in $CHCl_3$/EtOAc (4.0:1.0). Furthermore, δ_c values for the three methoxy carbons in 5,6,7-trimethoxy-3-(4′-hydroxybenzyl)-4-chromanone were observed at 61.8, 61.5 and 56.3. The down-field shifts of the two carbons indicating that two of the methoxy groups are attached to a carbon with *ortho*-disubstitutents. On the other hand, the ^{13}C NMR of **6** displayed only one down-shifted resonance signal for one of the methoxy groups at δ_c 61.2. The HREIMS of compound **6** showed a molecular ion at M^+/z 344.1269 (calc. 344.1259) corresponding to a molecular formula of $C_{19}H_{20}O_6$. It also showed the presence of a fragment ion at m/z 107 indicative of only one –OH substituent in ring B (4′-OH). The three methoxy groups in compound **6** were, therefore, deduced to be located at C-5, C-7 and C-8. The UV spectrum showed no change upon addition of both $AlCl_3$/HCl and NaOAc confirming lack of free OH at the C-5 and C-7 positions.

2.2. 3-Benzyl-3-hydroxy-4-chromanones *8* and *9*

The ^{1}H NMR spectra of **8** and **9** were characterized by the presence of two sets of doublets between δ 4.1–4.3 and 2.6–3.1 for the protons at C-2 and C-9. The absence of a complex multiplet at ca. δ 2.8, as was observed in the ^{1}H NMR spectra of **1**–**7** was an indication that C-3 was substituted, in this case by an hydroxyl group (vide infra). The ^{13}C NMR spectrum was similar to that of **1**, except for the absence of the quaternary carbon signal at 46.2 and the appearance of a new signal at 71.7. The ^{1}H NMR spectrum also showed the presence of an AA′BB′ system for ring B and two *meta* coupled proton signals for ring A. In addition, the spectrum of **9** showed a methoxy signal at δ 3.79. The location of the methoxy group was deduced to be at C-4′ from MS data which showed an ion at m/z 121 (m/z 107 for compound **8**) due to cleavage of the C3–C9 benzylic bond. The foregoing spectroscopic data allowed the assignment of the two compounds to the structures shown in **8** and **9**. These compounds are known to occur in *E. bicolor* (Farkas et al., 1970) and S. African *Scilla* species (Broadbent et al., 1975), respectively.

2.3. Scillascillin homoisoflavonoids *10* and *11*

Compounds **10** and **11** were optically active ($[\alpha]_D^{25}$ + 56 and + 60, respectively) and their molecular formulas were determined as $C_{18}H_{16}O_6$ and $C_{19}H_{18}O_6$, respectively, from HREIMS data 328.0949 (calc. 328.0946) and 342.1109 (calc. 342.1103), respectively. The ^{1}H NMR spectra of **10** (Table 3) showed two sets of doublets resonating at δ 4.50/4.56 (2H, *d*, J = 11.3 Hz) and 3.04/3.54 (2H, *d*, J = 13.4 Hz) for protons at C-2 and C-9, respectively. Two broad singlets at δ 5.79 and 5.81 were assigned to the C-6 and C-8 protons in ring A. Consideration of the rings and double bonds deduced from the molecular formula and analysis of the ^{13}C data led us to conclude that compounds **10** and **11** belonged to the scillascillin type of homoisoflavonoids (Heller and Tamm, 1981). Two singlet signals in the ^{1}H NMR spectrum at δ 6.86 and 6.76 were assigned to two protons located *para* to each other in ring B. Two methoxy signals were observed, and the location of these groups were deduced to be at C-3′ and C4′ on the basis of the observed HMBC and NOE correlations. Important NOE and HMBC correlations are shown in Fig. 1. The ^{1}H NMR spectrum of **11** was similar to compound **10** for the protons at C-2, C-9, C-2′ and C-5′. The spectrum also showed *meta* coupled proton signals at δ 6.08 (1H, *d*, J = 2.3 Hz) and 6.10 (1H, *d*, J = 2.3 Hz) which were assigned to H-6 and H-8 protons, respectively. Compound **11** further showed the presence of three methoxy signals, two of which were assigned to C-3′ and C-4′. The third methoxy was deduced to be at C-7 on the basis of UV data using shift reagents, the presence of a chelated OH signal in the ^{1}H NMR spectrum and HMBC correlations (Experimental).

Table 3
^{1}H NMR spectral data, δ (J Hz) for compounds **8**, **9**, **11** in CD_3COCD_3 and **10** in CD_3OD (300 MHz)

	8	**9**	**10**	**11**
H-2a	4.29, *d*, (11.4)	4.07, *d*, (11.1)	4.56, *d*, (11.3)	4.68, *d*, (11.4)
H-2b	4.11, *d*, (11.4)	4.02, *d*, (11.3)	4.50, *d*, (1 1.2)	4.64, *d*, (11.4)
H-6	5.90	5.95	5.79	6.08, *d*, (2.3)
H-8	5.90	5.95	5.81	6.10, *d*, (2.3)
H-9a	3.12, *d*, (14.0)	2.99, *d*, (14.0)	3.54, *d*, (13.4)	3.56, *d*, (13.4)
H-9b	2.67, *d*, (14.0)	2.92, *d*, (14.1)	3.04, *d*, (13.4)	3.06, *d*, (13.4)
H-2′	7.12, *d*, (8.5)	7.23, *d*, (8.5)	6.89	6.89
H-3′	6.81, *d*, (8.5)	6.87, *d*, (8.5)	–	–
H-5′	6.81, *d*, (8.5)	6.87, *d*, (8.5)	6.73	6.76
H-6′	7.12, *d*, (8.5)	7.23, *d*, (8.5)	–	–
5-OH	–	–	–	12.17
7-OMe	–	–	–	3.89
3′-OMe	–	–	3.83	3.81
4′-OMe	–	3.78	3.77	3.73

2.4. Xanthones *12* and *13*

The structures of xanthones **12** and **13** were deduced from spectroscopic data. Both compounds are known from natural sources although not from higher plants. Xanthone **12** has been found in *Lecanora reuteri* and *Penicillium patulum* (Broadbent et al., 1975) and has

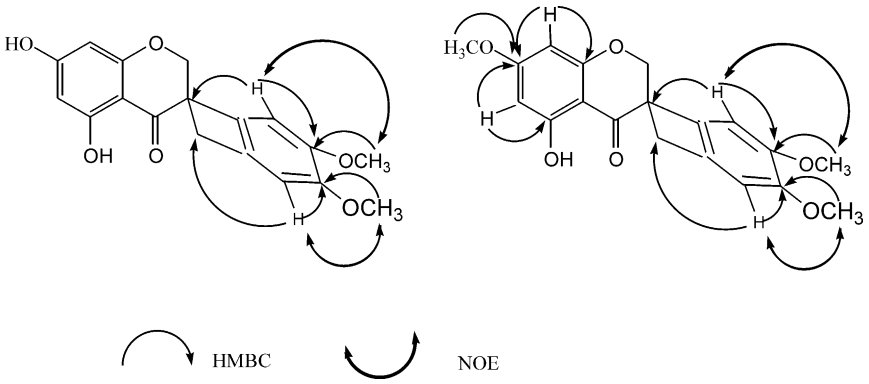


Fig. 1. HMBC and NOE correlations for compounds **10** and **11**.

been synthesized subsequently (Sandifer et al., 1981; Sundholm, 1978). The ^{1}H NMR and other spectroscopic data reported for this compound were practically identical to those measured in this study. Xanthone **13** has been reported also from *P. patulum* and *Aspergillus versicolor* (McMaster et al., 1960; Kingston et al., 1976) but neither these authors nor the references quoted by them (MacMillan, 1953; Grove et al., 1952) for this compound provide any NMR data. The report referred to above for **12** also does not give any ^{13}C NMR data. The full spectroscopic data and signal assignments have now been made for **12** and **13** (Experimental). Although Xanthones are known to occur in a few of the higher plant families such as *Guttiferae* and *Gentianaceae*, they are commonly found in fungi and lichens (Bennet and Hiok-Huang, 1989; Peres and Nagem, 1997). Our finding of these compounds in *Ledebouria* is remarkable and we were initially uncertain if they were derived from a fungal or lichen contaminant. Our suspicion was strengthened by the realization that 8-methyl substituted xanthones are restricted to fungi and lichens (Peres and Nagem, 1997). In order to rule out such a possibility we purchased and re-examined fresh specimens of *L. graminifolia*. Although direct TLC comparison of crude extract with authentic specimens was not conclusive, a combination of preparative and analytical TLC procedures (see Experimental) were conducted to unequivocally establish the presence of both xanthones. Further proof of presence of xanthones **12** and**13** was obtained by examination of constituents of bulbs of in vitro regenerated *L. graminifolia*. In vitro regeneration of *L. graminifolia* was achieved using twin scale and leaf scale techniques on Murashige–Skoog (MS) (Murashige and Skoog, 1962) medium supplemented with 3% sucrose, 1% agar, 2.0 mg/l benzyladenine (BA) and 0.2 mg/l naphthalene acetic acid (NAA). Mature *L. graminifolia* plants were obtained with bulbs that are morphologically similar to the wild ones. The bulbs from in vitro regenerated plants were phytochemically investigated (Experimental) and TLC comparison with authentic compounds **1–13**, showed that the cultured bulbs contained all the 13 compounds and in particular, we were able to clearly identify the presence of xanthones **12** and **13**. We, therefore, conclude that they are genuine metabolites of *L. graminifolia*.

Samples of the new homoisoflavonoids characterized in this study (**3**, **5**,**6**, **10** and **11**) were submitted to the US National Cancer Institute for in vitro primary cytotoxic and antiprolferative activity using a panel of 60 different human tumor cell lines (Boyd and Paul, 1995. Compound **5** was noted to show some activity (GI_{50} at 7 μg/ml, on MCF7 breast cancer line and the IC_{50} of 13, 16, and 30 be by far the most active among them and it showed further selectivity in its action on MCF7 on MDA-MB-435, MDA-N and Hs578 T breast cancer cell lines, respectively). The other compounds were either inactive at the concentrations tested or less active by at least a factor of 10.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 300 for proton and at 75 MHz for carbon, respectively. δ values are expressed as ppm. Low Resolution Mass Spectroscopy (LRMS) were obtained on Finnigan Mat SSQ 7000 Single Quadrupole MS using a direct sample inlet via a heated solid probe, or on a Finnigan LCQDECA mass spectrometer into which a solution of the sample [dissolved in H_2O–CH_3CN (1:1) containing 25 mM formic acid] is introduced to the ion source by means of a syringe pump at ca. 50 μl/ml for APCI and at ca. 5 μl/ml, for ESI. HREIMS were run by D.P Boshoff of the Mass Spectroscopy Unit, Cape Technikon, Cape Town, SA. The UV spectra were measured in MeOH, on a Shimadzu UV-2101PC spectrophotometer. Infrared (IR) spectra were measured as KBr pellets or in $CHCl_3$ on Perkin Elmer System 2000 Ft-IR spectrometer. Mps were done on a Griffin Melting Point Instrument and are uncorrected. Flash Chromatography (FC) was carried out in a 55 cm×4 cm column packed with silica gel 60 (particle size

0.040–0.063 mm). Gel filtration using Sephadex LH-20 was eluted with $CHCl_3$:MeOH (2:1). TLC was done on ready-made TLC aluminum plates (20×20 cm) coated with silica gel 60 F_{254} (0.25 mm thick). The resulting spots were observed using a UV lamp at 254 nm and sprayed using vanillin–H_2SO_4. Preparative TLC plates (0.5 mm thick) were prepared using either Silica gel 60 $HF_{254+366}$ for thin layer chromatography (Merck, Germany) or Silica gel 60 PF_{254} containing $CaSO_4$ for preparative layer chromatography (Merck, Germany) on 20×20 cm glass plates.

3.2. Plant material

The bulbs of *L. graminifolia* were purchased from a stall owned by Mrs. B. Chaba at the Gaborone Railway Station, Botswana. Two of the bulbs were grown in our Experimental Garden to obtain botanical specimens for identification. Identification was done by Dr. L.M. Turton and later confirmed by Mr. G. Pope of the Royal Botanical Garden at Kew, UK. A voucher specimen, Lg11601, was deposited at the Herbarium, Department of Biological Sciences, University of Botswana.

3.3. Plant material and culture methods

L. graminifolia bulbs were purchased from the Gaborone Station vendors. The roots and dead scale leaves were carefully removed from bulbs after which the bulbs were washed with running tap water. This was followed by sterilization with Benlate (2%) fungicide (Crouch et al., 1999b) in a warm bath at 45–50 °C and rinsing three times with sterile water to minimize contamination by soil fungi. The bulbs were further sterilized in 3% sodium hypochlorite solution with Tween 20 for 30 min followed by subsequent rinsing in sterile water before processing. In vitro regeneration of *L. graminifolia* was achieved using twin scale and leaf scale techniques on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose, 1% agar, 2.0 mg/l benzyladenine (BA) and 0.2 mg/l naphthalene acetic acid (NAA). The cultures were incubated and maintained at 25 °C, 16 h light/8 h dark photoperiod regime provided by cool white fluorescent tubes at 40 μmol^{-1} m s^{-1} light intensity. After an 8-week incubation period, the shoots (with no roots) produced were transferred for rooting into a basic MS medium with no hormone supplements. The rooted plantlets were then transferred into the green house for acclimatization.

3.4. Extraction and isolation

The bulbs purchased from vendors were chopped into pieces, dried and ground, yielding 100 g of powdered plant material, which was soaked for 24 h at room temp. in CH_2Cl_2:MeOH (1:1) followed by MeOH (200 ml) for about 30 min. The two extracts were combined and freed of solvent under reduced pressure, to yield an organic residue of 7.6 g, which was adsorbed on silica gel (15 g) and loaded onto a column packed with silica gel (80 g) using petrol. The column was eluted successively with petrol containing ethyl acetate gradient and frs of about 250 ml each were collected as follows: fr 1 (100% petrol), fr 2–3 (20% EtOAc), fr 4–6 (50% EtOAc), fr 7–8 (100% EtOAc), fr 9 (10% MeOH–EtOAc) and fr 10 (20% MeOH–EtOAc). TLC showed that fr 8, 9 and 10 contained same compounds therefore they were combined.

Fr 4 (ca. 500 mg) was subjected to Sephadex LH-20 and six frs, 25 ml each, were collected. PTLC of fr 5 in $CHCl_3$:MeOH (98:2) gave compound **11** (5.1 mg). Fr 6 was submitted to PTLC using $CHCl_3$:MeOH (95:5) as the eluent yielding six bands of which band IV was further applied on to PTLC in $CHCl_3$:EtOAc (95:5) affording compound **4** (8.8 mg) and **13** (4.8 mg). Band VI was subjected to PTLC in $CHCl_3$ yielding compound **10** (3.8 mg).

Fr 5 (2 g), 6 (700 mg) and 7 (1.3 g) were each subjected to Sephadex LH-20 followed by PTLC in $CHCl_3$:MeOH (95:5). Fr 5 afforded compounds **1** (79 mg), **2** (15.6 mg), **12** (2.7 mg), **8** (8.3 mg) and **9** (68 mg); fr 6, compound **7** (1.7 mg) whilst fr 7 gave **3** (100 mg) and **6** (4.8 mg).

The combined frs 8–10 were submitted to PTLC and eluted with $CHCl_3$:MeOH (95:5) giving three bands. Band 3 was further subjected to PTLC in $CHCl_3$:MeOH (95:5) and afforded compound **5**.

3.5. Re-examination of presence of xanthones *12* and *13*

Two fresh bulbs of *L. graminifolia*, purchased from the Gaborone Station, were chopped, dried and ground affording 3.23 g of powdered plant material. Extraction with CH_2Cl_2:MeOH (1:1) followed by neat MeOH yielded 180 mg of organic extract. Analytical TLC [$CHCl_3$:MeOH (96:4)] of the organic extract and comparison with authentic compounds **12** and **13** was inconclusive since spots of several compounds appeared at the same R_f as those of **12** and **13**. Therefore, the organic extract was subjected to PTLC in $CHCl_3$:MeOH (95:5) yielding five major bands. Analytical TLC of band 1 and 4 in $CHCl_3$: EtOAc (96:4) clearly showed the presence of xanthones **12** and **13**, respectively.

3.6. Chromatographic analysis of in vitro regenerated bulbs

Four fresh in vitro regenerated cultured bulbs were subjected to routine extraction procedures yielding 783 mg of organic extract. The organic extract was adsorbed on silica gel and subjected to flash chromatography on silica gel eluting with petrol containing increasing

amounts of EtOAc. Fractions of about 250 ml were collected giving a total of five fractions: fr 1 (20% EtOAc), fr 2 (50% EtOAc), fr 3 (100% EtOAc), fr 4 (10% MeOH in EtOAc) and fr 5 (20% MeOH in EtOAc). Fractions 1–5 were analyzed by analytical TLC comparison with compounds **1**–**13** previously obtained from the wild bulbs of *L. graminifolia*. Analytical TLC showed that the compounds were mostly found in fr 2 although they were not well resolved. Fr 2 was therefore subjected to PTLC giving seven bands which were then analyzed by TLC in comparison with the authentic samples. Xanthones **12** and **13** were observed in bands 1 and 5, respectively.

*3.7. 5-Hydroxy-7-methoxy-3-(4′-hydroxybenzyl)-4-chromanone (**3**)*

White powder: mp 160–162 °C; $[\alpha]_D^{25}$ −54.6 (MeOH, *c* 0.78); UV λ_{max} $^{(MeOH)}$ nm (log ε): 205 (7.37), 208 (7.39), 224 (7.34), 285 (7.24); $\lambda_{max}^{(MeOH+NaOMe)}$ nm (log ε): 207 (7.38), 225 (7.34), 284 (7.25), 365 (5.44); $\lambda_{max}^{(MeOH+AlCl_3)}$ nm (log ε): 208 (7.38), 225 (7.34), 284 (7.25), 365 (5.44); $\lambda_{max}^{(MeOH+AlCl_3+HCl)}$ nm (log ε): no change; $\lambda_{max}^{(MeOH+NaOAc)}$ nm (log ε): 208 (7.50), 288 (7.05), 320 (7.23); IR ν_{max} (KBr) cm^{-1}: 3264 (–OH), 1653, 1605 (C=O), 1514, 1459, 1385, 1289, 1251, 1162, 1125, 963, 833; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; LRESIMS *m*/*z* 301 [M + H] $^+$ (100), 283 (5), 193 (17), 133 (9), 167 (33), 107 (19), 77 (3); HREIMS *m*/*z* 300.1012 (calc. for $C_{17}H_{16}O_5$: 300.0997).

*3.8. 5-Hydroxy-6,7-dimethoxy-3-(4′-hydroxybenzyl)-4-chromanone (**5**)*

Yellow powder: mp 74–76 °C (dec.); $[\alpha]_D^{25}$ +345.3 (MeOH, *c* 0.43); UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 204 (7.46), 212 (7.46), 279 (7.29), 343 (6.83); $\lambda_{max}^{(MeOH+NaOMe)}$ nm (log ε): 208 (7.73), 248 (7.49), 291 (7.22), 390 (6.74); $\lambda_{max}^{(MeOH+AlCl_3)}$ nm (log ε): 204 (7.46), 214 (7.46), 279 (7.28), 343 (6.81); $\lambda_{max}^{(MeOH+AlCl_3+HCl)}$ nm (log ε): no change; $\lambda_{max}^{(MeOH+NaOAc)}$ nm (log ε): 207 (7.75), 278 (7.29), 341 (6.49); IR ν_{max} ($CHCl_3$) cm^{-1}: 3321, 2939, 1672, 1615, 1498, 1453, 1230, 1198, 1104; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; LREIMS *m*/*z* 330 [M$^+$] (100), 299 (8), 223 (34), 196 (34), 153 (16), 107 (32); HREIMS *m*/*z* 330.1103 (calc. for $C_{18}H_{18}O_6$: 330.1103).

*3.9. 5,7,8-Trimethoxy-3-(4′-hydroxybenzyl)-4-chromanone (**6**)*

Yellow gum; UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 232 (5.93), 286 (5.97), 324 (5.46); $\lambda_{max}^{(MeOH+NaOMe)}$ nm (log ε): 206 (6.17), 239 (6.09), 285 (5.96), 325 (5.60); $\lambda_{max}^{(MeOH+AlCl_3)}$ nm (log ε): 232 (5.95), 285 (5.97); $\lambda_{max}^{(MeOH+AlCl_3+HCl)}$ nm (log ε): no change; $\lambda_{max}^{(MeOH+NaOAc)}$ nm (log ε): 207 (6.25), 226 (6.00), 285 (5.96), 325 (5.53); IR $\acute{\imath}_{max}$ ($CHCl_3$) cm^{-1}: 3315, 2933, 1673, 1601, 1510, 1464, 1368, 1131, 981; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; LRESIMS *m*/*z* 345 [M + H] $^+$ (83), 239 (100), 223 (58), 209 (40), 180 (8), 153 (3), 107 (3); HREIMS *m*/*z* 344.1269 (calc. for $C_{19}H_{20}O_6$: 344.1259).

*3.10. 3,5,7-Trihydroxy-3-(4′-methoxybenzyl)-4-chromanone (**9**)*

Brown gum; UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 202 (7.80), 214 (6.97), 293 (6.76); $\lambda_{max}^{(MeOH\ +\ NaOMe)}$ nm (log ε): 207 (7.35), 326 (6.81); $\lambda_{max}^{(MeOH\ +\ AlCl_3)}$ nm (log ε): 201 (6.85), 224 (6.77), 316 (6.60), 377 (5.76); $\lambda_{max}^{(MeOH\ +\ AlCl_3\ +\ HCl)}$ nm (log ε): no change; λ_{max} $^{(MeOH\ +\ NaOAc)}$ nm (log ε): 205 (7.29); IR ν_{max} ($CHCl_3$) cm^{-1}: 3207, 2934, 1638, 1510, 1169, 835; ^{1}H NMR, Table 3; ^{13}C NMR, Table 2; EIMS *m*/*z* 316 [M$^+$] (18), 195 (4), 153 (2), 121 (100), 87 (6), 58 (22).

*3.11. 5,7-Dihydroxy-3′, 4′-dimethoxyspiro{2H-1-benzopyran-7′-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one (**10**)*

Yellow gum: $[\alpha]_D^{25}$ +56.0 (MeOH, *c* 0.035); UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 234 (6.51), 293 (6.82); $\lambda_{max}^{(MeOH\ +\ AlCl_3)}$ nm (log ε): 235 (6.53), 313 (6.89); $\lambda_{max}^{(MeOH+AlCl_3+HCl)}$ nm (log ε): 235 (6.52), 313 (6.90), 366 (6.02); $\lambda_{max}^{(MeOH+NaOAc)}$ nm (log ε): 210 (7.47), 328 (6.86); IR ν_{max} ($CHCl_3$) cm^{-1}: 3464, 3020, 1633, 1216, 929; ^{1}H NMR, Table 3; ^{13}C NMR, Table 2; APCI *m*/*z* 329 $[M+H]^+$ (100), 314 (27), 297 (18), 269 (18); HREIMS *m*/*z* 328.0949 (calc. for $C_{16}H_{16}O_6$: 328.0946).

*3.12. 5-Hydroxy-3′,4′,7-trimethoxyspiro{2H-1-benzopyran-7′-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one (**11**)*

White powder: mp 150–153 °C; $[\alpha]_D^{25}$ +60.0 (MeOH, *c* 0.033); UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 203 (7.02), 291 (6.83); $\lambda_{max}^{(MeOH\ +\ NaOMe)}$nm (log ε): no change; $\lambda_{max}^{(MeOH+AlCl_3)}$ nm (log ε): 203 (7.08), 225 (6.94), 313 (6.93), 381 (6.10); $\lambda_{max}^{(MeOH\ +\ AlCl_3+HCl)}$ nm (log ε): no change; $\lambda_{max}^{(MeOH\ +\ NaOAc)}$ nm (log ε): no change; IR ν_{max} (KBr) cm^{-1}: 3421, 2920, 1639, 1297, 1209, 838; ^{1}H NMR, Table 3; ^{13}C NMR Table 2; LRESIMS *m*/*z* 343 [M + H] $^+$ (100), 328 (27), 311 (17), 283 (25), 177 (2), 151 (5); HREIMS *m*/*z* 342.1109 (calc. for $C_{19}H_{18}O_6$: 342.1103).

*3.13. 1,3,6-Trihydroxy-8-methylxanthone (**12**)*

Yellow powder; mp 226–228 °C [Lit. 274–275 °C (Broadbent et al., 1975)]; UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 241 (6.70), 310 (6.47); $\lambda_{max}^{(MeOH+AlCl_3)}$ nm (log ε): 202 (6.77), 233 (6.58), 250 (6.51), 261 (6.51), 339 (6.58), 382 (6.16); $\lambda_{max}^{(MeOH+AlCl_3\ +\ HCl)}$ nm (log ε): no change; IR ν_{max} (KBr) cm^{-1}: 3482, 2917, 1650, 1622, 1302, 1180, 830; ^{1}H NMR (CD_3COCD_3): 13.60 (1H, *s*, H-1), 6.16 (1H, *d*,

$J = 1.9$ Hz, H-2), 6.30 (1H, *d*, $J = 1.9$ Hz, H-4), 6.67 (2H, H-5, H-7), 2.77 (3H, *s*, 8-CH_3); ^{13}C NMR (CD_3COCD_3): 157.6 (C-1), 102.8 (C-1a), 98.3 (C-2), 165.9 (C-3), 93.6 (C-4), 164.9 (C-4a), 101.0 (C-5), 160.0 (C-5a), 164.1 (C-6), 117.1 (C-7), 143.4 (C-8), 111.3 (C-8a), 182.1 (C-9), 23.0 (8-CH_3); EIMS *m*/*z* 258 [M^+] (100), 229 (9), 149 (6), 115 (3), 69 (4), 57 (6).

3.14. 1,6-Dihydroxy-3-methoxy-8-methylxanthone (**13**)

Yellow powder: mp 210–212 °C (dec.); UV $\lambda_{max}^{(MeOH)}$ nm (log ε): 241 (6.36), 309 (6.16); $\lambda_{max}^{(MeOH\ +\ AlCl_3)}$ nm (log ε): 239 (6.16), 253 (6.20), 261 (6.19), 339 (6.28), 382 (5.81); $\lambda_{max}^{(MeOH + AlCl_3 + HCl)}$ nm (log ε): no change; IR ν_{max} (KBr) cm^{-1}: 3423, 2917, 1651, 1605, 1298, 1168, 826; ^1H NMR (CD_3COCD_3): 13.58 (chelated –OH), 6.24 (1H, *d*, $J = 2.3$ Hz, H-2), 6.40 (1H, *d*, $J = 2.3$ Hz, H–4), 6.70 (2H, H-5, H-7), 2.78 (3H, *s*, 8-CH_3), 3.91 (3H, 3-OCH_3). ^{13}C NMR (CD_3COCD_3): 156.5 (C-1), 102.9 (C-1a), 96.0 (C-2), 165.4 (C-3), 91.1 (C-4), 164.1 (C-4a), 100.1 (C-5), 159.1 (C-5a), 162.9 (C-6), 116.3 (C-7), 142.7 (C-8), 110.4 (C-8a), 181.4 (C-9), 22.8 (8-CH_3), 54.8 (3-OCH_3); APCI *m*/*z* 273 [M + H]$^+$ (100), 258 (1.8), 245 (0.2), 230 (0.3), 213 (0.5), 146 (6).

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References

Adinolfi, M., Barone, G., Lanzetta, R., Laonigro, G., Mangoni, L., Parrilli, M., 1985. Three 3-benzyl-4-chromanones from *Muscari comosum*. Phytochemistry 24, 624–626.

Bennet, G.J., Hiok-Huang, L., 1989. Xanthones from Guttiferae. Phytochemistry 28, 967–998.

Boyd, M.R., Paul, K.D., 1995. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. Drug Develop. Res. 34, 91–109.

Broadbent, D., Mabelis, R.P., Spencer, H., 1975. 3,6,8-Trihydroxy-1-methylxanthone: an antibacterial metabolite from *Penicillium patulum*. Phytochemistry 4, 2082–2083.

Crouch, N.R., Bangani, V., Mulholland, D.A., 1999a. Homoisoflavonoids from three South African *Scilla* species. Phytochemistry 51, 943–946.

Crouch, N.R., Brunkhost, M., McCartan, S.A., 1999b. Micropropagation of *Scilla nervosa* (Hyacinthaceae), a Southern African medicinal bulb. S. Afri. J. Bot. 65, 306–307.

Farkas, L., Gottsegen, A., Nogradi, M., 1970. The synthesis of Eucomin and (±)-Eucomol. Tetrahedron 26, 2787–2790.

Grove, J.F., Macmillan, J., Mulholland, T.P.C., Rogers, M.A.T., 1952. Griseofulvin I. J. Chem. Soc. 3949.

Heller, W., Andermatt, P., Schaad, W.A., Tamm, C., 1976. 213 Homoisoflavanone, IV. Neue Inhaltstoffe der Eucomin-Reihe von *Eucomis bicolor*. Helv. Chim. Acta 59, 2048–2056.

Heller, W., Tamm, C., 1981. Homoisoflavanones and biogenetically related compounds. Forschritte der Chemie Organischer Naturstoffe 40, 105.

Hutchings, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.

Kingston, D.G.I., Chen, P.N., Vercellotti, J.R., 1976. Metabolites of *Aspergillus versicolor*: 6,8-di-*O*-methylnidurufin, griseofulvin, dechlorogriseofluvin, and 3,8-dihydroxy-6-methoxy-1-methylxanthone. Phytochemistry 15, 1037–1039.

MacMillan, J., 1953. Griseofulvin VII. Dechlorogriseofulvin. J. Chem. Soc. 1702–1967.

McMaster, W.J., Scott, A.I., Trippett, S., 1960. Metabolic products of *Penicillium patulum*. J. Chem. Soc. 4629.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15, 473–497.

Peres, V., Nagem, T.J., 1997. Trioxygenated naturally occurring xanthones. Phytochemistry 44, 191–214.

Pohl, T.S., Crouch, N.R., Mulholland, D.A., 2000. Southern African Hyacinthaceae: chemistry, bioactivity and ethnobotany. Curr. Org. Chem. 4, 1287–1324.

Sandifer, R.M., Bhattacharya, A.K., Harris, T., 1981. Acylation of multiple anions of poly-β-ketones by hydroxy- and alkoxybenzoates. Cyclization of the resultant tetraketones to benzophenones and xanthones. J. Org. Chem. 46, 2260–2267.

Silayo, A., Ngadjui, B.T., Abegaz, B.M., 1999. Homoisoflavonoids and stilbenes from the bulbs of *Scilla nervosa subsp. rigidifolia*. Phytochemistry 52, 947–955.

Sundholm, E.G., 1978. Total synthesis of lichen xanthones. Tetrahedron 34, 577–586.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, pp. 714–716.