SHORT PAPER

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Chromone studies. Part 11.[1] Synthesis and electron-impact mass spectrometric study of granulosin and side-chain analogues[†]

Perry T. Kaye*, Aloysius T. Nchinda and, in part, Christopher A. Gray

Department of Chemistry, Rhodes University, Grahamstown, 6140, South Africa

Granulosin, a chromone constituent of the bark of *Galipea granulosa*, and four analogues, all of which exhibit toxicity to the brine shrimp *Artemia salina*, have been prepared from 2′,3′,4′-trihydroxyacetophenone. High-resolution mass spectrometric analysis has permitted elucidation of the fragmentation patterns exhibited by these systems following electron-impact ionisation.

Keywords: granulosin, chromone, electron-impact ionisation

A number of naturally occurring chromones are known to be biologically active. Notable amongst these are the furochromone, khellin,[2] which has found use in the treatment of bronchial asthma, and the cytotoxic styrylchromone, hormothamnione.[3] Schiff and co-workers,[4] in their investigation of Costa Rican medicinal plants, have recently reported the isolation of a chromone derivative, granulosin **6c**, from the bark of *Galipea granulosa*. We have been engaged in an ongoing study of chromone systems[1] and, in this communication, we report on:- (i) the efficient synthesis of granulosin and four structural analogues; (ii) their toxicity to the brine shrimp *Artemia salina*; and (iii) an investigation of their electron-impact mass fragmentation patterns.

The first step of the synthesis involved formation of the dioxolane derivative **2** *via* regioselective acetalisation of 2′,3′,4′-trihydroxyacetophenone **1** (Scheme 1) using bromochloromethane in the presence of caesium carbonate.[5] The strong intramolecular hydrogen-bonding between the 2′-hydroxyl and acetyl carbonyl groups in 2′-hydroxyacetophenones was expected to inhibit reaction of the 2′-hydroxyl group and favour selective acetalisation of the 3′- and 4′-hydroxyl groups in 2′,3′,4′-trihydroxyacetophenone **1**. However, in an initial reaction, using 1.5 equivalents of bromochloromethane, the expected acetal **2** was obtained in only 12% yield; the major product (88%) was found to be the "dimeric" system **3**, which arose from the reaction of all three phenolic hydroxyl goups. When the proportion of bromochloromethane was limited to 1 equivalent, the reaction proceeded smoothly to afford the required 2′-hydroxy-3′,4′-(methylenedioxy)-acetophenone **2** in 80% yield.

Treatment of 2′-hydroxy-3′,4′-(methylenedioxy)acetophenone **2** with two equivalents of sodium ethoxide in ethanol afforded the enolate which, on reaction with a series of ethyl carboxylate esters [R = CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $(CH_3)_2CH$, $PhCH_2$] gave mixtures, indicated by ^1H NMR spectroscopy to contain the corresponding acylated products (existing, in each case, as an enol tautomer, formulated as structure **4**) and their cyclised derivatives **5**. Treatment of these mixtures with a mixture of acetic and sulfuric acid afforded the chromone derivatives **6a–e** in yields ranging from 58 to 85%. The product structures were confirmed by elemental (HREIMS) and spectroscopic (IR, ^1H and ^{13}C NMR) analysis, and the data obtained for the 2-propyl derivative **6c** were shown to correspond closely to those reported [4] for granulosin (chemical shifts lie within 0.03-0.19 ppm for the ^1H- and 0.1–1.1 ppm for the ^{13}C NMR spectra in DMSO-d_6).

All five of the chromone derivatives **6a–e** showed significant cytotoxic effects against the brine shrimp *Artemia salina*. LC_{50} values estimated by probit analysis[6] (Table 1) indicate an interesting range in activity with granulosin **6c** being highly toxic (LC_{50} : 4 ppm), compounds **6b** and **6d** moderately toxic (LC_{50} : 22 and 21 ppm respectively) and compounds **6a** and **6e** least toxic (LC_{50} : 132 and 109 ppm respectively).

Table 1 Summary of *A. salina* assay data: Estimates of median lethal concentrations obtained by probit analysis[6]

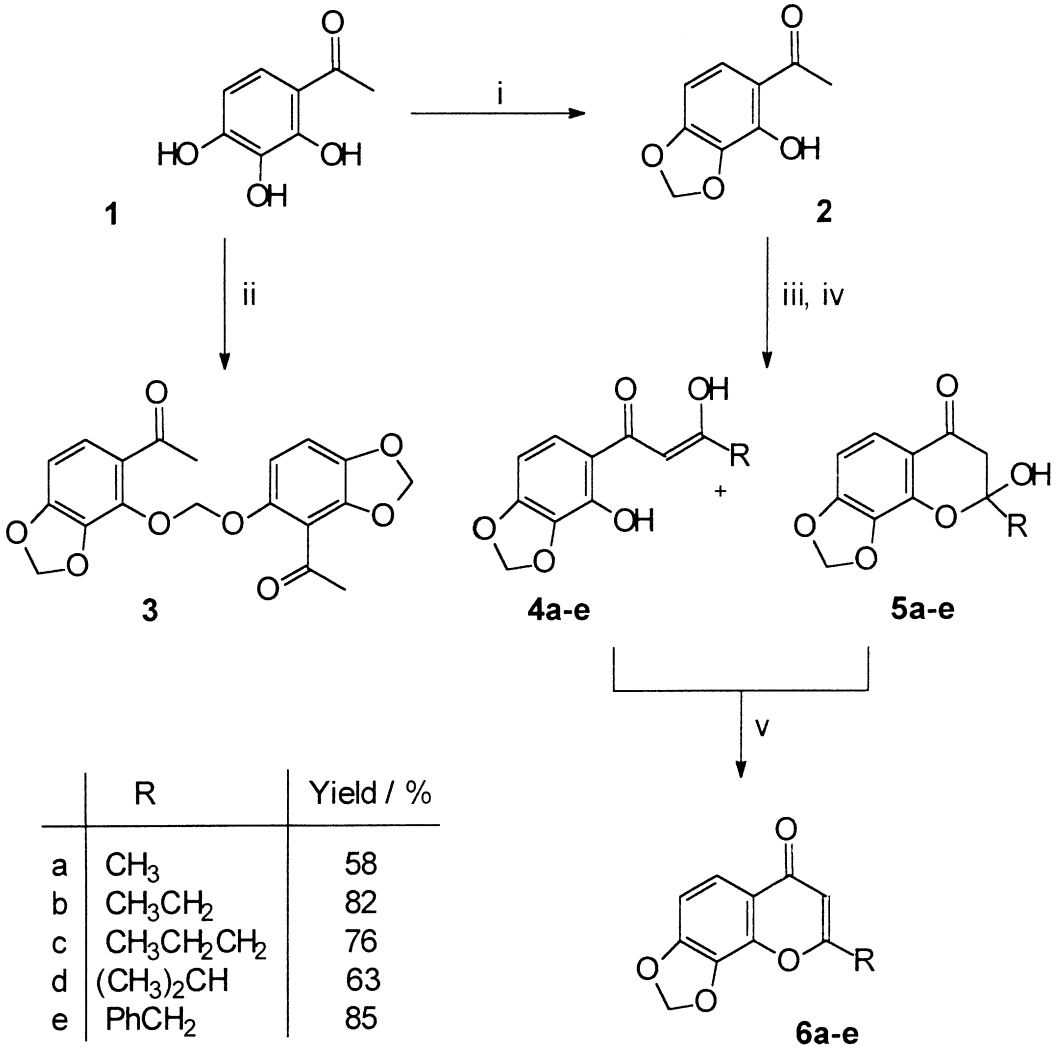
	LC50 (μg/ml)	95 % Confidence intervals (μg/ml)	
6a	131.8	112.6	156.3
6b	22.3	19.8	25.0
6c	4.3	3.5	5.2
6d	21.3	18.2	24.7
6e	108.6	93.4	126.7

Significant fragmentation pathways in the mass spectra of the chromone derivatives **6a–e** were explored using high-resolution and B/E link-scan data. The five major fragmentations (paths A–E) observed for granulosin **6c** are illustrated in Scheme 2. Loss of H. from the molecular ion **Ic** (Path A) presumably reflects formation of the resonance-stabilised oxonium species **IIc**, the fragmentation being characteristic of 1,3-dioxolanes.[7] Elimination of a methyl radical from the molecular ion **Ic**, followed by a 1,2-hydride shift (path B) would account for the resonance-stabilised carbocation **IIIc** (*m*/*z* 217); the same fragment can form directly through loss of a methyl radical from the isomeric analogue **6d** (Fig. 1). Elimination of a hydrogen atom from the molecular ions for compounds **6a, 6b** and **6e** affords cations at *m*/*z* 203, 217 and 279, respectively; these *m*/*z* values could, however, correspond to either (or both) of the ion-types **II** or **III** (Fig. 1).

Chromone itself is known to undergo extrusion of CO to form an odd-electron benzofuranoid fragment,[8] and similar ring-contractions are evident in the mass spectra of the compounds studied here. In the case of granulosin **6c**, an initial McLafferty-type rearrangement (path C; Scheme 2) leads to the odd-electron species **IVc** which, on elimination of CO and H., affords an even-electron species (*m*/*z* 175), formulated as the resonance-stabilised benzofuran **Vc**. The sequence, **Ic** → **IVc** → **Vc**, is supported by the B/E link-scan data. However, none of the analogues, **6a,6b,6d** or **6e**, contain a γ-hydrogen and, thus, cannot undergo a McLafferty-type rearrangement. Consequently, formation of the corresponding benzofuranoid fragments, **Va,Vb,Vd** and **Ve**, appears to involve initial

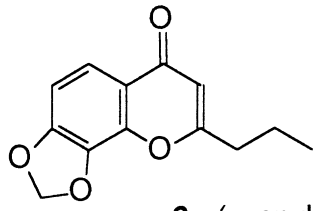
* To receive any correspondence. E-mail: P.Kaye@ru.ac.za

† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.



	R	Yield / %
a	CH_3	58
b	CH_3CH_2	82
c	$CH_3CH_2CH_2$	76
d	$(CH_3)_2CH$	63
e	$PhCH_2$	85

i) $BrCH_2Cl$ (1 eq.), Cs_2CO_3, DMF; ii) $BrCH_2Cl$ (1.5 eq.), Cs_2CO_3, DMF; iii) NaOEt - EtOH; iv) RCO_2Et; v) AcOH - H_2SO_4.

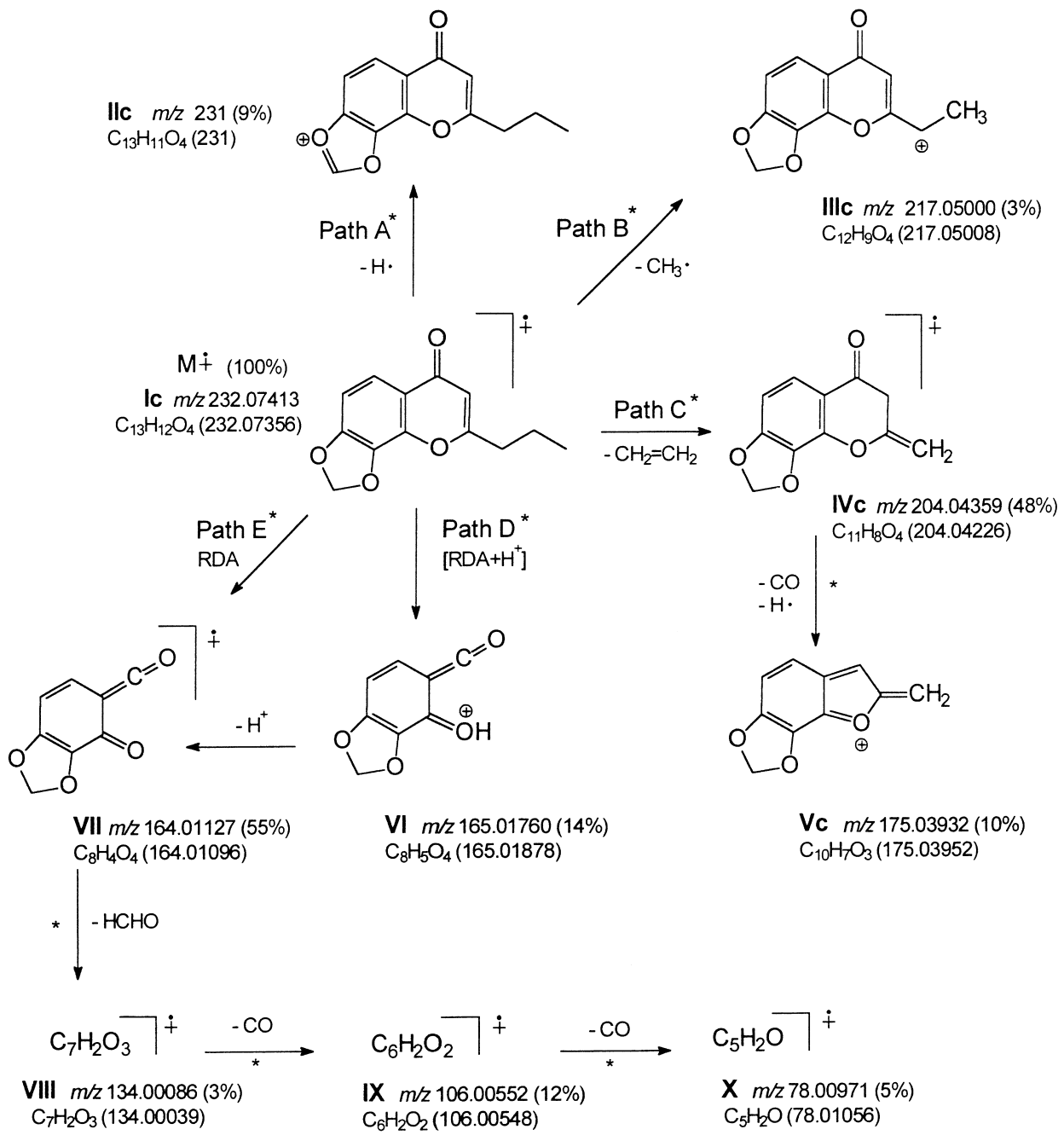


6c (granulosin)

Scheme 1

decarbonylation of the molecular ion, followed by elimination of a radical species ($R^1 \cdot$) from the corresponding intermediates **XI** (see Fig. 2). While 2-methylchromone has also been observed to afford an even-electron benzofuranoid fragment,[8] formation of the corresponding cations **Va–e** is, presumably, enhanced by the additional stabilisation arising from delocalisation of a lone pair on the distal dioxolane oxygen.

Chromones are also known[8] to undergo *retro*-Diels–Alder fragmentation – as reflected in the formation of the common, conjugated ketenes **VI** and **VII** (*via* paths D and E respectively; Scheme 2).[9] Elimination of H^+ (**VI** → **VII**) and the subsequent fragmentations (**VII** → **VIII** → **IX** → **X**) are all supported by the link-scan data. In addition to the expected *retro*-Diels–Alder fragments, **VI**(*m/z* 165) and **VII**(*m/z* 164),



Scheme 2

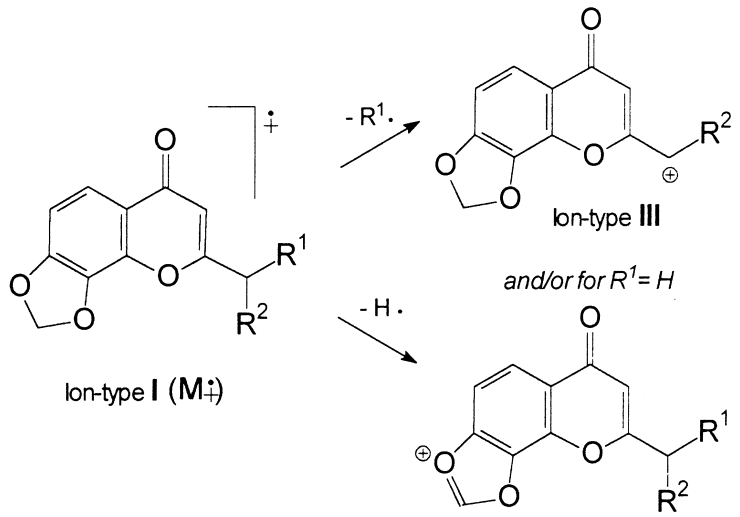
Major fragmentation pathways in the EI mass spectrum of granulosin **6c**. *m/z* values are followed, in parentheses, by the % relative abundance, and atomic compositions by the calculated mass. An asterisk indicates a fragmentation supported by B/E link-scan data.

the 2-benzyl derivative **6e** also affords a fragment at *m/z* 115, which corresponds to the well-stabilised benzylic-propargylic cation **XIIe** (Fig. 2) resulting from an alternative *retro*-Diels–Alder pathway. Under the ionising conditions used, the molecular ion was, in all cases, the base peak.

Experimental

IR spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker Avance 400MHz NMR spectrometer and are referenced using the solvent signals. Low-resolution mass spectra were recorded on a Finnigan-Mat GCQ mass spectrometer, and high resolution mass spectra were obtained on a VG70-SEQ double-focusing magnetic sector instrument by Dr P. Boshoff at the Mass Spectrometry Unit, Cape Technikon, Cape Town.

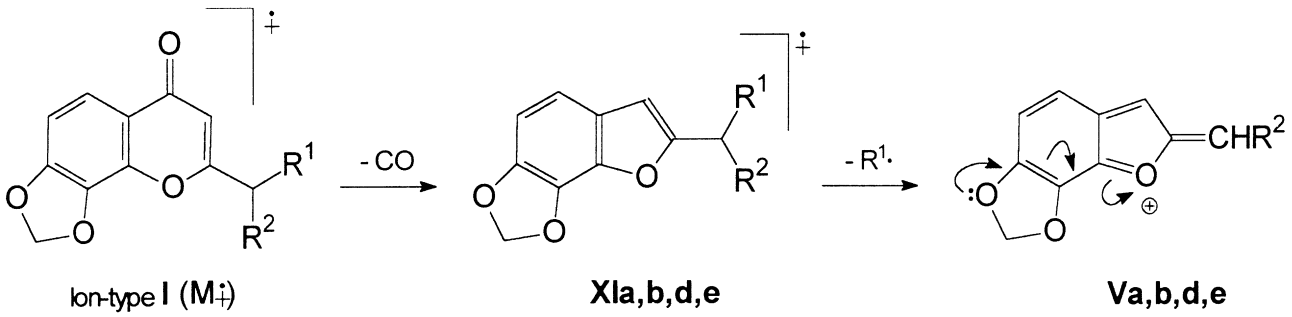
2′-Hydroxy-3′,4′-(methylenedioxy)acetophenone 2:To a mechanically stirred suspension of 2′,3′,4′-trihydroxyacetophenone (0.5 g, 3.0 mmol) and Cs_2CO_3 (0.97g , 3.0 mmol) in dry DMF (7.5 ml) was added $BrCH_2Cl$ (0.20 ml, 3.0 mmol), and the resulting mixture was boiled under reflux. After 3h, the mixture was allowed to cool to room temperature and then filtered through a pad of Celite 545, washing with EtOAc. The filtrate and washings were concentrated almost to dryness, and the residue diluted with H_2O (20 ml) and extracted with EtOAc (3 × 50 ml). The combined extracts were washed with water (25 ml) and then with brine (25 ml), and dried over anhydrous $MgSO_4$. Evaporation of the solvent give a dark-tan solid, which was recrystallised from petroleum ether (b.p. 80–100°C) to afford *2′-hydroxy-3′,4′-(methylenedioxy)acetophenone* **1** as a pale yellow solid

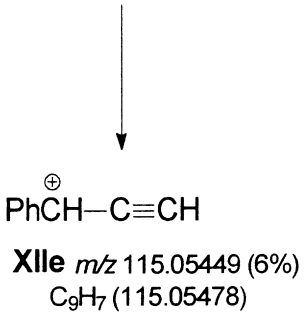


Compd.	R^1	R^2	*M/z* [a]
6a	H	H	203(29)[b]
6b	H	CH_3	217(21)[b]
6d	CH_3	CH_3	217(3)
6e	H	Ph	279(9)[b]

[a] *M/z* for corresponding ion-type **III**; followed, in parentheses, by % relative abundance. Fragmentations supported by high-resolution and link-scan data.
[b] Ion types **II** and **III** have the same *m/z*.

Fig. 1 Common fragmentations to afford type **III** and/or type **II** cations.





Compd.	R^1	R^2	*M/z* for ion-types[a] XI	V
6a	H	H	176(6)	175(2)[b]
6b	H	CH_3	190(2)	189(<1)[b,c]
6d	CH_3	CH_3	204(<2)[d]	189(8)
6e	H	Ph	252(<1)	251(2)[b]

[a] Followed, in parentheses, by % relative abundance. Fragmentations supported by high-resolution and link-scan data.
[b] Loss of H · from the dioxolane ring would afford fragment with the same *m/z*.
[c] Loss of R^2 · gives cation, *m/z* 175 (4%).
[d] Peak shoulder corresponds to $C_{12}H_{12}O_3$.

Fig. 2 Alternative fragmentations exhibited by the side-chain analogues **6a,b,d,e.**

(0.42 g, 80%), m.p. 96–98°C (Found: M^+, 180.04259. $C_9H_8O_4$ requires *M*, 180.04226); γ_{max} (KBr)/cm^{-1} 1663 (CO); δ_H(400 MHz; $CDCl_3$) 2.56 (3H, s, CH_3), 6.07 (2H, s, CH_2), 6.46 (1H, d, *J*=8.4 Hz, 5′-H), 7.37 (1H, d, *J*=8.5 Hz, 6′-H) and 12.27 (1H, s, OH); δ_C(100 MHz; $CDCl_3$) 27.0 (CH_3), 101.2 (C-5′), 103.0 (CH_2), 117.4 (C-1′), 127.0 (C-6′),134.9 (C-3′), 147.4 (C-2′), 154.4 (C-4′) and 203.8 (C=O).

Bis[6-acetyl-2,3-(methylenedioxy)phenoxy]methane 3: The experimental procedure employed for the synthesis of 2′-hydroxy-3′,4′-(methylenedioxy) acetophenone **2** was followed, using 2′,3′,4′-trihydroxyacetophenone (1.0g, 6.0 mmol), Cs_2CO_3 (2.93g, 9.0 mmol), dry DMF (15 ml) and $BrCH_2Cl$ (0.60 ml, 9.0 mmol). After heating for 2h, the reaction mixture was worked up to afford a yellow-brown solid. Flash chromatography [on silica gel : elution with hexane-EtOAc (1:1)] gave, as a white crystalline solid, *bis[6-acetyl-2,3-(methylenedioxy)phenoxy]methane* **3** (1.96 g, 88%), m.p. 133–135°C (Found: M^+, 372.08495. $C_{19}H_{16}O_8$ requires *M*, 372.08452); γ_{max} (KBr)/cm^{-1} 1665 (CO); δ_H(400 MHz, $CDCl_3$), 2.38 (6H, s, CH_3), 5.95 (4H, s, 2×CH_2), 6.09 (2H, s, CH_2), 6.60 (2H, d, *J*=8.3 Hz, 5′-H) and 7.32 (2H, d, *J* 8.3=Hz, 6′-H); δ_C (100 MHz, $CDCl_3$) 30.8 (CH_3), 94.3 and 102.0 (2×CH_2), 104.0 (C-5′), 125.5 (C-6′), 126.5 (C-1′), 137.2 (C-3′), 139.2 (C-2′), 152.4 (C-4′) and 197.3 (C=O).

The procedures used for the synthesis of granulosin **6c** and its analogues **6a,b,d,e** are illustrated by the following examples.

7,8-(Methylenedioxy)-2-propylchromone (granulosin) **6c:** A mixture of 2′-hydroxy-3′,4′-(methylenedioxy)acetophenone **2** (0.50 g, 2.77 mmol) and $CH_3CH_2CH_2CO_2Et$ (1.6 ml, 12 mmol) was added dropwise to a stirred dispersion of NaOEt [generated *in situ* by adding Na metal (0.27g, 11.6 mmol) to dry EtOH (2.0 ml)]. The resulting dark-green mixture was boiled gently under reflux for 8h, during which time, a thick yellow slurry was formed. After cooling, the reaction mixture was poured into Et_2O (15 ml) and, after standing for 2h, the sodium salt was filtered off, washed with Et_2O and dissolved in ice-cold water (15 ml). The resulting solution was acidified with acetic acid, and then extracted with Et_2O (3 × 25 ml); the combined ethereal extracts were dried with anhydrous $MgSO_4$ and evaporated *in vacuo* afforded a brick-red residue indicated, by ^{1}H NMR spectroscopy, to contain a mixture of 6-[2′-hydroxy-3′,4′-(methylenedioxy)phenyl]-4,6-hexanedione (as an enol tautomer, formulated as **4c**) and 2-hydroxy-7,8-(methylenedioxy)-2-propylchromanone **5c**, which was used without further purification. The crude mixture, together with glacial acetic acid (4.0 ml) and conc. H_2SO_4 (0.1 ml), was boiled under reflux for 4 hours. The hot solution was poured into ice-cold water (20 ml), the mixture basified with 10% aq. $NaHCO_3$ (20ml), and the resulting dark-purple precipitate filtered and washed with cold water. Flash chromatography of the precipitate [on silica gel; elution with hexane-EtOAc (1:1)] afforded a colourless solid, which was recrystallised from petroleum ether (b.p. 80–100°C) – methanol (1:1) to afford, as colourless crystals, 7,8-(methylenedioxy)-2-propylchromone **6c** (0.48g, 76%), m.p. 101–103°C (lit.[4], 102–103°C) (Found: M$^+$, 232.07413. Calc. for $C_{13}H_{12}O_4$ M, 232.07356); γ_{max} (KBr)/cm^{-1} 1658 (CO); δ_H (400 MHz; DMSO-d_6) 0.96 (3H, t, *J*=7.4 Hz, CH_3), 1.68 (2H, m, CH_3CH_2), 2.61 (2H, t, *J*=7.4 Hz, $CH_3CH_2CH_2$), 6.12 (1H, s, 3-H), 6.27 (2H,s, OCH_2O), 7.09 (1H, d, *J*=8.4 Hz, 6-H) and 7.56 (1H, d, *J*=8.4 Hz, 5-H); δ_C (100MHz; DMSO-d_6) 13.3 (CH_3), 19.7 (CH_3CH_2), 35.1 ($CH_3CH_2CH_2$), 103.6 (OCH_2O), 107.1 (C-6), 109.0 (C-3), 119.3 (C-4a and C-5; 119.8 and 120.1 in $CDCl_3$), 134.4 (C-8), 140.8 (C-8a), 152.1 (C-7), 168.8 (C-2) and 175.9 (C=O).

Analytical data for the new, granulosin analogues are as follows.

2-Methyl-7,8-(methylenedioxy)chromone **6a**: as a white crystalline solid (58%), m.p. 161–163°C [from petroleum ether (b.p. 80–100°C)] (Found: M$^+$, 204.04226. $C_{11}H_8O_4$ requires *M*, 204.04226); γ_{max} (KBr)/cm^{-1} 1657 (CO); δ_H(400 MHz; $CDCl_3$) 2.36 (3H, s, CH_3), 6.06 (1H, s, 3-H), 6.15 (2H, s, OCH_2O), 6.90 (1H, d, *J*=8.4 Hz, 6-H) and 7.74 (1H, d, *J*=8.4 Hz, 5-H); δ_C(100 MHz; $CDCl_3$) 20.3 (C-1′), 103.1 (OCH_2O), 106.9 (C-6), 110.1 (C-3), 119.7 (C-4a), 120.2 (C-5), 134.3 (C-8), 141.4 (C-8a), 152.1 (C-7), 165.3 (C-2) and 177.1 (C=O).

2-Ethyl-7,8-(methylenedioxy)chromone **6b***:* as a white crystalline solid (82%), m.p. 106–107°C [from petroleum ether (b.p. 80–100°C)] (Found: M$^+$, 218.05884. $C_{12}H_{10}O_4$ requires *M*, 218.05791); γ_{max} (KBr)/cm^{-1} 1653 (CO); δ_H(400 MHz; $CDCl_3$) 1.29 (3H, t, *J* =7.5 Hz, CH_3), 2.63 (2H, m, CH_3CH_2), 6.06 (1H, s, 3-H), 6.15 (2H, s, OCH_2O), 6.89 (1H, d, *J*=8.5 Hz, 6-H) and 7.73 (1H, d, *J*=8.5 Hz, 5-H); δ_C(100 MHz; $CDCl_3$) 10.9 (C-2′), 27.2 (C-1′), 103.1 (OCH_2O), 106.8 (C-6), 108.4 (C-3), 119.8 (C-4a), 120.1 (C-5), 134.4 (C-8), 141.4 (C-8a), 152.1 (C-7) 169.9 (C-2) and 177.3 (C=O).

2-Isopropyl-7,8-(methylenedioxy)chromone **6d**: as a brown crystalline solid (63%), m.p. 119–121°C [from petroleum ether (b.p. 80–100°C)] (Found: M$^+$, 232.07364. $C_{13}H_{12}O_4$ requires *M*, 232.07356); γ_{max} (KBr)/cm^{-1} 1634 (CO); δ_H(400 MHz; $CDCl_3$) 1.31 (6H, d, *J*=6.7 Hz, 2×CH_3), 2.85 (1H, m, CH), 6.09 (1H, s, 3-H), 6.16 (2H, s, OCH_2O), 6.90 (1H, d, *J*=8.3 Hz, 6-H) and 7.74 (1H, d, *J*=8.3 Hz, 5-H); δ_C(100 MHz; $CDCl_3$) 20.1 (C-2′), 33.1 (C-1′), 103.1 (OCH_2O), 106.8 (C-6), 107.0 (C-3), 119.8 (C-4a), 120.1 (C-5), 134.4 (C-8), 141.4 (C-8a), 152.1 (C-7), 173.4 (C-2) and 177.6 (C=O).

2-Benzyl-7,8-(methylenedioxy)chromone **6e**: as a white crystalline solid (85%), m.p. 172–174°C [from petroleum ether (b.p. 80–100°C)] (Found: M$^+$, 280.07415. $C_{17}H_{12}O_4$ requires *M*, 280.07356); γ_{max} (KBr)/cm^{-1} 1657 (CO); δ_H(400 MHz; $CDCl_3$) 3.90 (2H, s, CH_2Ph), 6.00 (1H, s, 3-H), 6.l4 (2H, s, OCH_2O), 6.88 (1H, d, *J*=8.5 Hz, 6-H), 7.25–7.34 (5H, m, Ar-H) and 7.72 (1H, d, *J*=8.4 Hz, 5-H); δ_C(100 MHz; $CDCl_3$) 40.4 (CH_2Ph), 103.1 (OCH_2O), 106.9 (C-6), 110.2 (C-3), 119.7 (C-4a), 120.2 (C-5), 127.5, 128.9, 129.3 and 134.5 (Ar-C), 134.6 (C-8), 141.4 (C-8a), 152.2 (C-7), 167.3 (C-2) and 177.2 (C=O).

Assessment of biological activity: Artemia salina larvicidal bioassays were performed as described by Solis *et al.*[10] Estimates of median lethal concentrations were obtained by probit analysis[6] of *A. salina* mortality data from 12 solutions across concentration ranges of 25.00 - 0.586 μg/ml for **6c**, and 400.0–12.50 μg/ml for **6a, 6b, 6d** and **6e**.

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References

1 P.T. Kaye, A.T. Nchinda, L.V. Sabbagh and C. McCleland, *J. Chem. Res*., 2000, 237-239.
2 J.K. Landquist, in *Comprehensive Heterocyclic Chemistry*, Ed. O. Meth-Cohn, Pergamon Press, Oxford, 1984, Vol. 1, p. 151.
3 W.H. Gerwick, A. Lopez, G.D. Van Duyne, J. Clardy, W. Ortiz and A. Baez, *Tetrahedron Lett*., 1996, **27**, 1979-1982.
4 J.A. López, W. Barillas, J. Gomez-Laurito, G.E. Martin, A.J. Al-Rehaily, M.A. Zemaitis and P.L. Schiff, Jr., *J. Nat. Prod*., 1997, **60**, 24-26.
5 R.E. Zelle and W.J. McClellan, *Tetrahedron Lett*., 1991, **32** (22), 2461-2464.
6 D.J. Finney, in *Probit Analysis* (3rd edn), Cambridge University Press, Cambridge, 1971, p. 333ff.
7 A.J. Elliot, in *Comprehensive Heterocyclic Chemistry*, ed. K.I. Potts, Pergamon Press, Oxford, 1984, Vol. 6, p. 756.
8 P.J. Brogden, C.D. Gabbutt and J.D. Hepworth, in *Comprehensive Heterocyclic Chemistry*, eds. A.J. Boulton and A. McKillop, Pergamon Press, Oxford, 1984, Vol. 3, p. 613
9 The B/E link-scan data indicate that the direct fragmentations, ***M*** → **VI** (*m*/*z* 165), ***M*** → **VII** (*m*/*z* 164) and ***M***–1 → **VII** (*m*/*z* 164), are typically favoured. In the case of granulosin **6c**, however, it is apparent that these are complemented by the fragmentations, **IVc** → **VI** (*m*/*z* 165) and **IVc** → **VII** (*m*/*z* 164).
10 P.N. Solis, C.W. Wright, M.M. Anderson, M.P.Gupta and J.D. Phillipson, *Planta Med*., 1993, **59**, 250-252.