Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2505–2511

Synthesis and structural revision of naturally occurring ayapin derivatives

Dominick Maes,[a] Stijn Vervisch,[a] Silvia Debenedetti,[b] Carlos Davio,[c] Sven Mangelinckx,[a] Nicola Giubellina[a] and Norbert De Kimpe[a,*]

[a]*Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium*
[b]*Department of Biological Sciences, Faculty of Sciences, National University of La Plata, Calle 115 and 47, 1900 La Plata, Argentina*
[c]*Laboratory of Radioisotopes, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, 1113 Buenos Aires, Argentina*

Received 24 September 2004; revised 20 December 2004; accepted 22 December 2004

Available online 26 January 2005

Abstract—The synthesis of three highly oxygenated naturally occurring coumarins, 8-methoxy-6,7-methylenedioxycoumarin, 5-methoxy-6,7-methylenedioxycoumarin and 5,8-dimethoxy-6,7-methylenedioxycoumarin is described for the first time, together with a new method for the preparation of ayapin (6,7-methylenedioxycoumarin). Comparison of the spectroscopic data of the synthetic tetraoxygenated coumarin 5,8-dimethoxy-6,7-methylenedioxycoumarin with literature reports resulted in the structural revision of several natural coumarins. Two coumarins, both identified as 5,8-dimethoxy-6,7-methylenedioxycoumarin must have other structures, while the structure of another coumarin, described as the isomeric 7,8-dimethoxy-5,6-methylenedioxycoumarin has to be revised to 5,8-dimethoxy-6,7-methylenedioxycoumarin.

1. Introduction

In the last decade, a large number of tri- and tetraoxygenated coumarins have been isolated from plants. Examples include purpurenol,[1] purpurasol,[2] purpurasolol,[3] isopurpurasol,[4] 8-methoxy-6,7-methylenedioxycoumarin **2**,[5] 5-methoxy-6,7-methylenedioxycoumarin **3**[6,7] and 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** (Fig. 1). In this article we report the synthesis of four coumarins bearing a methylenedioxy substituent at C6 and C7. Hence they are all derivatives of 6,7-methylenedioxycoumarin **1** (ayapin). 6,7-Methylenedioxycoumarin **1** was first isolated from *Eupatorium ayapana* (Asteraceae)[8] and was given the trivial name ayapin. Later, ayapin **1** was also isolated from a number of other plants, including *Helianthus annuus*,[9] *Artemisia apiacea*,[10] *Pterocaulon virgatum*[11] and *P. polystachyum*.[12]

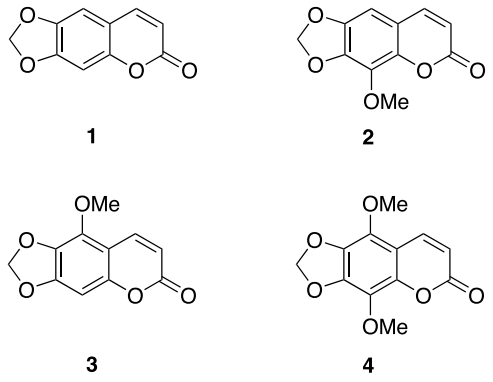


Figure 1.

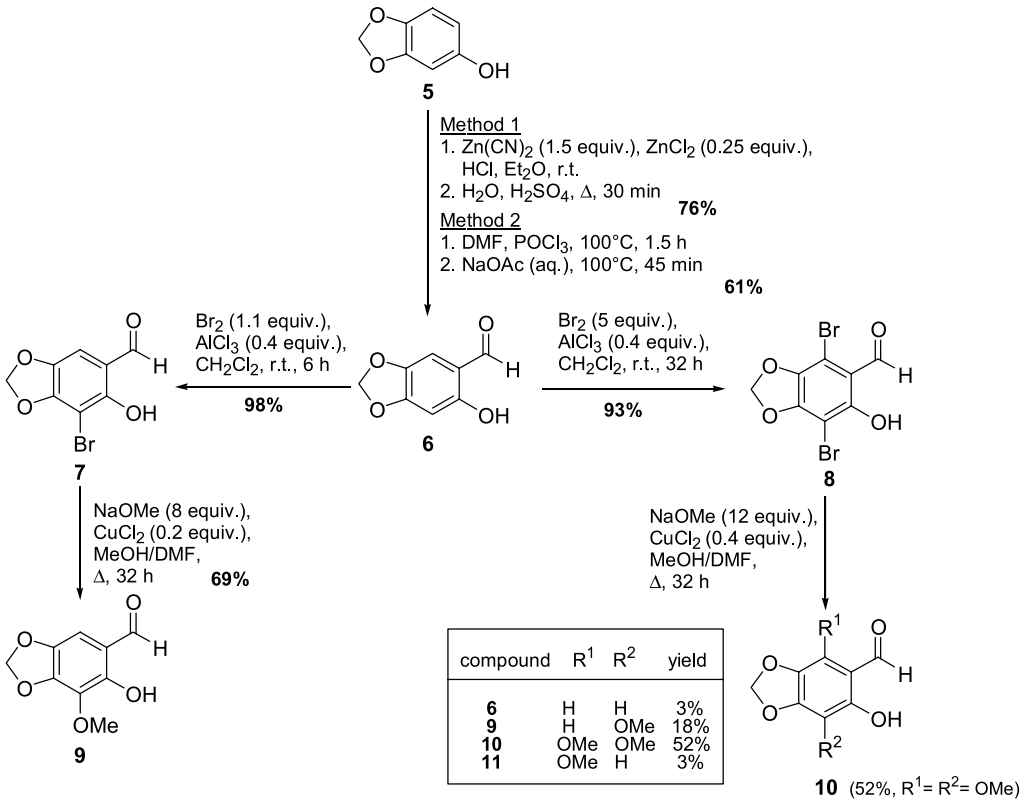
2. Results and discussion

Different methods for the synthesis of coumarins from *o*-hydroxybenzaldehydes were described in the literature. A convenient way for the synthesis of coumarins is the reaction of salicylaldehydes with alkoxycarbonylmethylenephosphorane in *N*,*N*-diethylaniline under reflux.[13–16] Although this method has been successfully applied for the synthesis of simple mono- and dioxygenated coumarins,[13–16] this is the first report on its application on tri- and tetraoxygenated coumarins. The key feature of the synthetic pathway was the synthesis of suitable *o*-hydroxybenzaldehydes which then are transformed to the corresponding coumarins via the Wittig reaction.

2-Hydroxy-4,5-methylenedioxybenzaldehyde **6** was

Keywords: Coumarins; *o*-Hydroxybenzaldehydes; Wittig reaction; Natural products; Structural revision; 6*H*-[1,3]Dioxolo[4,5-*g*]chromen-6-ones.
* Corresponding author. Tel.: +32 9 264 59 51; fax: +32 9 264 62 43; e-mail: norbert.dekimpe@UGent.be

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.12.061



Scheme 1.

synthesized from sesamol **5** in two different ways (Scheme 1).[18] Using the Gattermann formylation,[17,18] 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** was obtained in 76% yield. The Villsmeier–Haack reaction[18,19] yielded 61% of the desired aldehyde **6**. Different conditions for the bromination of compound **6** were evaluated. Treatment of 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** with 2 equiv of bromine in dichloromethane resulted in no reaction. Low yields of 3-bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **7** were obtained using bromine in acetic acid. Best results were obtained when the reaction was performed in dichloromethane with aluminium(III) chloride as a Lewis catalyst. Thus, when 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** was stirred for 6 h with 1.1 equiv of bromine and 0.4 equiv of aluminium(III) chloride, 3-bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **7** was obtained in excellent yield (98%). Interestingly, it was found that when more equivalents of bromine were used and longer reaction times were applied, also the dibrominated benzaldehyde **8** was present in the reaction mixture. This was remarkable since it involved bromination at the deactivated *ortho* position to the carbonyl. Optimization of the latter side reaction by applying a large excess of bromine (5 equiv), 0.4 equiv of aluminium(III) chloride and an extended reaction time of 32 h resulted in a high yielding procedure for the synthesis of 3,5-dibromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **8** (93%).

The next step involved the nucleophilic aromatic substitution of these arylbromides with sodium methoxide. The reaction of arylhalides with sodium methoxide in the presence of copper(I) halides or pseudohalides as a catalyst has been described and different mechanisms have been postulated.[20–22] A problem that often occurs in this reaction is that not only the substitution reaction takes place but also the undesired reductive dehalogenation. This reductive dehalogenation is probably due to the formation of copper(0) in the reaction mixture.[21] It has been proven that polar co-solvents can improve the rate as well as the final yield of the reaction, most probably because these solvents increase the stability and solubility of the copper salts, which have poor solubility in pure methanol.[21–22] Amides such as *N*,*N*-dimethylformamide are sometimes used as co-solvents and have been useful in the substitution of aromatic aldehydes.[23,24] In order to optimize the reaction, the catalytic influence of different copper salts under the same reaction conditions was compared (Table 1). When no catalyst was added, no reaction could be observed (Table 1, entry 1). Different copper(I) salts such as copper(I) iodide, copper(I) bromide and copper(I) cyanide gave comparable results (Table 1, entries 2–4). Copper(II) hydroxide and basic copper(II) carbonate gave only complex reaction mixtures. Copper(II) acetoacetate an copper(II) acetate were lacking any catalytic activity (Table 1, entries 5 and 6). Good results were obtained using copper(II) chloride as a catalyst (Table 1, entry 9). Under the given circumstances, after 14 h, 60% of the desired 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9** was formed in the reaction mixture. Further optimization of the reaction procedure by using 8 equiv of sodium methoxide, 0.2 equiv of catalyst and a reaction time of 32 h gave 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9** in 69% isolated yield (Table 1, entry 10).

Table 1. Comparison of the catalytic activity of different copper salts for the synthesis of 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9** from 3-bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **7** (analysis of the reaction mixture based on ^{1}H NMR)

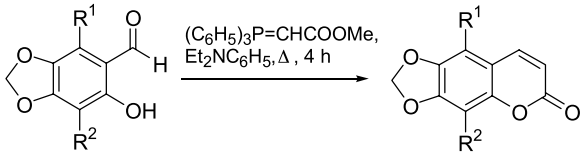
Entry		**7**	**6**	**9**
1[a]	No catalyst	100	0	0
2[a]	CuI	60	7	33
3[a]	CuBr	62	6	32
4[a]	CuCN	66	6	28
5[a]	$Cu(OH)_2$	Complex reaction mixture		
6[a]	$Cu_2CO_3(OH)_2$	Complex reaction mixture		
7[a]	$Cu(CH_3COO)_2$	100	0	0
8[a]	$Cu(acac)_2$	100	0	0
9[a]	$CuCl_2$	35	5	60
10[b]	$CuCl_2$	6	17	77 (69[c])

[a] Reaction conditions: 2 N NaOMe (20 equiv), catalyst (0.5 equiv), MeOH/DMF (3/1), △, 14 h.
[b] Reaction conditions: 2 N NaOMe (8 equiv), catalyst (0.2 equiv), MeOH/DMF (3/1), △, 32 h.
[c] Isolated yield after purification of the reaction mixture by column chromatography.

In a similar way 2-hydroxy-3,6-dimethoxy-4,5-methylenedioxybenzaldehyde **10** was synthesized in 52% isolated yield from 3,6-dibromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **8** and sodium methoxide in MeOH/DMF in the presence of copper(II) chloride under reflux for 32 h. 2-Hydroxy-4,5-methylenedioxybenzaldehyde **6** (3%), 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9** (18%) and 2-hydroxy-6-methoxy-4,5-methylenedioxybenzaldehyde **11** (3%) were isolated as side products in this reaction (Scheme 1).

The *o*-hydroxybenzaldehydes **6**, **9**, **10** and **11** were converted to the corresponding coumarins using the Wittig reaction of methoxycarbonylmethylenetriphenylphosphorane in *N*,*N*-diethylaniline.[13–16] In this way ayapin **1**, 8-methoxyayapin **2**, 5-methoxyayapin **3** and 5,8-dimethoxyayapin **4** were synthesized in high yields (77–82%, Scheme 2). Special attention was paid to the optimization of the workup procedure. In an earlier report we described a workup procedure that involved a crystallization from the reaction mixture.[25] An improved method involved the removal of the solvent through distillation under reduced pressure, followed by chromatography of the residue over silica gel. In this way high yields of the corresponding coumarins were obtained, as outlined in Scheme 2.

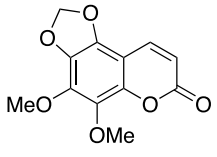
8-Methoxy-6,7-methylenedioxycoumarin **2** is a naturally occurring coumarin identified in *Asterolasia trymalioides* (Rutaceae), a shrub from South-East Australia.[5] Although the synthesis of 8-methoxy-6,7-methylenedioxycoumarin **2** from 6,7-dihydroxy-8-methoxycoumarin has been reported,[26] the published data for 8-methoxy-6,7-methylenedioxycoumarin do not correspond with those reported for the natural coumarin, nor with our own findings. However, the spectroscopic data we obtained for this coumarin are in full agreement with those published for the natural coumarin.[5]



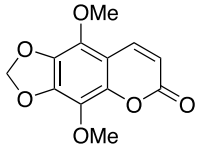
R^1	R^2	starting product	coumarin	yield
H	H	**6**	**1**	78 %
H	OMe	**9**	**2**	77 %
OMe	H	**11**	**3**	79 %
OMe	OMe	**10**	**4**	82 %

Scheme 2.

The isomeric 5-methoxy-6,7-methylenedioxycoumarin **3** was first isolated from *Simsia cronquistii* (Asteracea),[6] and from two *Pterocaulon* species, *P. virgatum* and *P. polystachyum*.[7] Very recently, it was found that 5-methoxy-6,7-methylenedioxycoumarin **3** is an inhibitor of cellular proliferation in human promyelocytic leukemia U-937 cells, and could therefore be a promising option for the treatment of several forms of leukemia.[27,28] 5,8-Dimethoxy-6,7-methylenedioxycoumarin **4** was reported twice in the literature.[29,31] Based on ^{1}H NMR and mass spectrometric data, 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** was proposed as the structure of sabandin, a natural coumarin isolated from *Ruta pinnata* (Rutaceae).[29,30] Recently, structure **4** was also assigned to artemicapin A, a coumarin from *Artemisia capillaris* (Asteraceae) based on ^{1}H NMR, ^{13}C NMR, NOESY and HMBC experiments.[31] Since the spectroscopic data from both references differ substantially, an unambiguous synthesis of this compound was desirable. The spectroscopic data for the synthesized 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** do not match with those reported for sabandin[29,30] or artemicapin A.[31] Moreover, the ^{13}C NMR and ^{1}H NMR correspond very well with those reported for a coumarin isolated from a Brazilian plant, *Metreodorea flavida* (Rutaceae).[32] This coumarin was identified as the regioisomeric structure 7,8-dimethoxy-5,6-methylenedioxycoumarin **12** (Fig. 2). Because no NOE correlation was found between the hydrogen at position 4 and the methoxy protons, the authors concluded that the methylenedioxy substituent was positioned at carbons 5 and 6.[32] Our own findings with the natural compound 5-methoxy-6,7-methylenedioxycoumarin **3**[6,7] revealed the absence of NOE correlation between H4



12



4

Figure 2.

Table 2. ^{1}H and ^{13}C NMR data for the natural coumarin from *Metreodorea flavida* and for 5,8-dimethoxy-6,7-methylenedioxycoumarin **4**

	Coumarin from *Metreodorea flavida*[32]	5,8-Dimethoxy-6,7-methylenedioxycoumarin
^{1}H NMR ($CDCl_3$)	3.99 (3H, s)	4.02 (3H, s)
	4.04 (3H, s)	4.06 (3H, s)
	6.01 (2H, s)	6.03 (2H, s)
	6.20 (1H, d, $J=9.7$ Hz)	6.22 (1H, d, $J=9.7$ Hz)
	7.91 (1H, d, $J=9.7$ Hz)	7.93 (1H, d, $J=9.7$ Hz)
^{13}C NMR ($CDCl_3$)	61.1 (–O*C*H_3)	60.3 (–O*C*H_3)
	61.1 (–O*C*H_3)	61.2 (–O*C*H_3)
	102.1 (–O*C*H_2O–)	102.2 (–O*C*H_2O–)
	107.0 (*C*q)	107.1 (*C*q)
	112.0 (*C*H)	112.2 (*C*H)
	126.9 (*C*q)	127.0 (*C*q)
	132.8 (*C*q)	132.9 (*C*q)
	133.4 (*C*q)	133.5 (*C*q)
	138.8 (*C*H)	138.9 (*C*H)
	142.9 (*C*q)	143.1 (*C*q)
	143.7 (*C*q)	143.8 (*C*q)
	160.5 (*C*=O)	160.6 (*C*=O)

and the protons of the methoxy substituent at C5. This was confirmed by the fact that no NOE correlation could be found between the H4 proton of the synthesized 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** and any of the methoxy groups. These observations prove that the presence or absence of a methoxy substituent at C5 cannot be derived from the results of NOE experiments.

Based on the spectroscopic evidence the structure of the natural coumarin from *M. flavida* has to be revised to 5,8-dimethoxy-6,7-methylenedioxycoumarin **4**. The ^{1}H NMR data for both the synthesized 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** and the natural coumarin from *M. flavida* are given in table 2. These data show clearly that the natural product from *M. flavida* is identical to 5,8-dimethoxy-6,7-methylenedioxycoumarin **4**.

3. Conclusion

Four natural coumarins were synthesized (of which three for the first time) from the corresponding *o*-hydroxybenzaldehydes using the Wittig reaction. Comparison of the spectroscopic data of the synthetic coumarins with literature data led to the structural revision of a tetraoxygenated coumarin from *M. flavida* and unequivocally proved the structure of a trioxygenated coumarin from *A. trymalioides*.

4. Experimental

4.1. General

^{1}H NMR spectra (270 or 300 MHz) and ^{13}C NMR spectra (68 or 75 MHz) were recorded on a Joel JNM-EX 270 NMR spectrometer or a Joel Eclipse FT 300 NMR spectrometer, respectively. IR spectra were recorded on a Perkin–Elmer Spectrum One spectrophotometer. Mass spectra were recorded on an Agilent 1100 Series VL mass spectrometer (ES 70 eV) or on a Varian MAT 112 mass spectrometer (EI 70 eV). Melting points were measured with a Büchi B-450 apparatus. Elemental analyses were measured with a Perkin–Elmer 2400 Elemental Analyzer. Flash chromatography was performed with ACROS silica gel (particle size 0.035–0.070 mm, pore diameter ca. 6 nm) using a glass column.

4.2. Synthetic procedures

4.2.1. 2-Hydroxy-4,5-methylenedioxybenzaldehyde 6. *Gattermann procedure*. Dry hydrogen chloride was bubbled through a stirred suspension of sesamol **5** (4.14 g; 30 mmol), zinc(II) cyanide (5.28 g, 45 mmol), zinc(II) chloride (1.02 g; 7.5 mmol) and a trace of sodium chloride in 100 ml of diethyl ether. To prevent the in situ formed hydrogen cyanide from escaping, the flask was connected to a condenser, cooled with ice water. After the formation of a green precipitate the solution is additionally treated with dry hydrogen chloride gas for 30 min. The ether was decanted and the precipitate was rinsed thoroughly with ether. The formed iminium salt was dissolved in 75 ml of water. A few drops of concentrated sulfuric acid were added and the resulting mixture was heated to 100 °C for 30 min. The reaction mixture was cooled to room temperature and the formed crystals were filtered off. The crystals were dissolved in dichloromethane and the solution was dried over magnesium sulfate. After filtration and evaporation of the solvent 3.76 g (76%) of pure 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** was obtained.

Vilsmeier procedure. Sesamol (4.14 g, 30 mmol) was dissolved in *N*,*N*-dimethylformamide (24 ml). At 0 °C 16 ml of phosphoroxytrichloride was added. The resulting mixture was stirred for 1 h at 100 °C. After cooling, the reaction mixture was poured into 250 ml of a saturated sodium acetate solution and heated for 45 min at 100 °C. The reaction mixture was cooled down again and the precipitate was filtered off. The precipitate was recrystallized from ethanol, giving 3.02 g (61%) of pure 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** as white needles.

Mp (°C): 127 (lit. 125–126[18]). IR (KBr, cm^{-1}): 3500 (broad, OH); 1605 (broad, C=O). ^{1}H NMR (270 MHz, $CDCl_3$): δ 6.02 (2H, s, O*C*H_2O); 6.46 (1H, s, 3-C*H*); 6.85 (1H, s, 6-C*H*); 9.62 (1H, s, C*H*O); 11.78 (O*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 98.33 (3-*C*H); 102.19 (O*C*H_2O); 109.34 (6-*C*H); 113.64 (1-C_q); 141.33 (5-C_q); 155.18 (2- or 4-C_q); 161.51 ((2- or 4-C_q); 193.71 (*C*HO). MS (70 eV,

EI, *m/z* (%)): 166 (M^+, 100); 165 (90); 107 (15); 79 (11); 69 (11); 53 (24); 52 (14); 51 (13). Anal. Calcd for $C_8H_6O_4$: C, 57.84%; H, 3.64%. Found: C, 57.99%; H, 3.49%.

4.2.2. 3-Bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde 7. To a solution of 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** (0.83 g; 5 mmol) and aluminium(III) chloride (0.27 g; 2 mmol) in dichloromethane (50 ml), bromine (0.88 g; 5.5 mmol) was added dropwise. After 6 h of stirring at room temperature the reaction mixture was poured into a saturated aqueous sodium metabisulfite solution (50 ml) and extracted three times with dichloromethane (50 ml). The combined organic layers were washed with 50 ml of a saturated sodium bicarbonate solution and 50 ml of water. The organic layer was dried over magnesium sulfate. After filtration and evaporation of the solvent 1.2 g (98%) of 3-bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **7** was obtained.

Mp (°C): 141 (light yellow solid). IR (KBr, cm^{-1}): 3440 (broad, OH); 1632 (C=O). ^1H NMR (270 MHz, $CDCl_3$): δ 6.12 (2H, s, OCH_2O); 6.87 (1H, s, 6-C*H*); 9.61 (1H, s, C*H*O); 12.36 (O*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 91.22 (*C*Br); 102.67 (O*C*H$_2$O); 108.59 (6-*C*H); 113.80 (1-C_q); 141.08 (5-C_q); 153.34 (2- or 4-C_q); 157.99 (2- or 4-C_q); 193.58 (*C*HO). MS (70 eV, EI, *m/z* (%)): 244/6 (M^+, 100); 243/5 (70); 131/3 (17); 79 (20); 77 (20); 55 (16); 53 (54); 51 (38); 50 (31); 49 (15); 44 (10). Anal. Calcd for $C_8H_5O_4Br$: C, 39.21%; H, 2.06%. Found: C, 39.37%; H, 2.15%.

4.2.3. 3,6-Dibromo-2-hydroxy-4,5-methylenedioxybenzaldehyde 8. To a solution of 2-hydroxy-4,5-methylenedioxybenzaldehyde **6** (0.83 g; 5 mmol) and aluminium(III) chloride (0.27 g; 2 mmol) in dichloromethane (50 ml) bromine (4 g; 25 mmol) was added dropwise. After 32 h of stirring at room temperature the reaction mixture was poured into a saturated sodium metabisulfite solution (50 ml) and extracted three times with dichloromethane (50 ml). The combined organic layers were washed with 50 ml of a saturated sodium bicarbonate solution and 50 ml of water. The organic layer was dried over magnesium sulfate. After filtration and evaporation of the solvent 1.51 g (93%) of 3,6-dibromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **8** was obtained.

Mp (°C): 198 (light yellow solid). IR (KBr, cm^{-1}): 3446 (broad, OH); 1627 (C=O). ^1H NMR (270 MHz, $CDCl_3$): δ 6.18 (2H, s, OCH_2O); 10.00 (1H, s, C*H*O); 13.42 (O*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 90.49 (3-*C*Br); 101.58 (6-*C*Br); 102.82 (O*C*H$_2$O); 110.83 (1-C_q); 139.85 (5-C_q); 152.83 (2- or 4-C_q); 160.27 (2- or 4-C_q); 194.37 (*C*HO). MS (70 eV, ES^-, *m/z* (%)): 321/323/325 ($M-H^+$). Anal. Calcd for $C_8H_4O_4Br_2$: C, 29.66%; H, 1.24%. Found: C, 29.79%; H, 1.32%.

4.2.4. 2-Hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde 9. 3-Bromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **7** (245 mg; 1 mmol) and copper(II) chloride (27 mg; 0.2 mmol) were dissolved in a mixture of 2 ml of methanol and 2 ml of *N*,*N*-dimethylformamide. To this solution 4 ml of a 2 N sodium methoxide solution in methanol was slowly added. The reaction mixture was stirred for 32 h at 100 °C. The solvent was removed under reduced pressure. The precipitate was dissolved in ether (20 ml), poured into 20 ml of a 2 N hydrogen chloride solution and extracted with diethyl ether (3×20 ml). The combined organic layers were washed with a 2 N hydrogen chloride solution (10 ml) and water (10 ml). The organic layer was dried over magnesium sulfate. After filtration and evaporation of the solvent the residue was chromatographed over silica gel (20% ethyl acetate/80% hexane), giving 136 mg (69%) of pure 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9**.

Mp (°C): 97.9–98.2 (white powder). IR (KBr, cm^{-1}): 3420 (broad, OH); 1620 (C=O). ^1H NMR (270 MHz, $CDCl_3$): δ 4.05 (3H, s, OCH_3); 6.02 (2H, s, OCH_2O); 6.65 (1H, s, 6-C*H*); 9.65 (1H, s, C*H*O); 11.73 (1H, s, O*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 60.50 (O*C*H$_3$); 102.32 (O*C*H$_2$O); 103.72 (6-*C*H); 114.03 (1-C_q); 141.92 (C_q); 144.63 (C_q); 144.96 (C_q); 153.55 (C_q); 194.16 (*C*HO). MS (70 eV, EI, *m/z* (%)): 196 (M^+, 100); 195 (19); 181 (11); 165 (16); 150 (16); 123 (10); 120 (13); 95 (13); 69 (13); 66 (11); 55 (24); 53 (21); 44 (10). Anal. Calcd for $C_9H_8O_5$: C, 55.11%; H, 4.11%. Found: C, 55.32%; H, 3.98%.

4.2.5. 2-Hydroxy-3,6-dimethoxy-4,5-methylenedioxybenzaldehyde 10. 3,6-Dibromo-2-hydroxy-4,5-methylenedioxybenzaldehyde **8** (0.97 g; 3 mmol) and copper(II) chloride (0.16 g; 1.2 mmol) were dissolved in a mixture of 12 ml of methanol and 12 ml of *N*,*N*-dimethylformamide. To this solution 24 ml of 2 N sodium methoxide in methanol was added dropwise. The reaction mixture was stirred for 32 h at 100 °C. The solvent was evaporated and the residue was dissolved in diethyl ether (120 ml), poured into 120 ml of 2 N hydrogen chloride solution and extracted with diethyl ether (3×100 ml). The combined extracts were washed with 2 N hydrogen chloride solution (60 ml) and water (60 ml). The organic layers were dried on magnesium sulfate. After filtration and evaporation of the solvent the residue was separated by chromatography over silica (20% ethyl acetate, 80% hexane). This procedure yielded 508 mg (52%) of 2-hydroxy-3,6-dimethoxy-4,5-methylenedioxybenzaldehyde **10**, 106 mg (18%) of 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9**, 18 mg (3%) of 2-hydroxy-6-methoxy-4,5-methylenedioxybenzaldehyde **11**, and 15 mg (3%) of 2-hydroxy-4,5-methylenedioxybenzaldehyde **6**.

2-Hydroxy-3,6-dimethoxy-4,5-methylenedioxybenzaldehyde **10**. Mp (°C): 121.4 (white powder). IR (KBr, cm^{-1}): 3447 (broad, OH); 1625 (C=O). ^1H NMR (270 MHz, $CDCl_3$): δ 3.94 (3H, s, 6-OCH_3); 4.07 (3H, s, 3-OCH_3); 5.97 (2H, s, OCH_2O); 10.04 (1H, s, C*H*O); 12.59 (1H, s, O*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 60.22 (O*C*H$_3$); 60.90 (O*C*H$_3$); 102.01 (O*C*H$_2$O); 107.46 (1-C_q); 126.72 (3-C_q or 5-C_q); 128.07 (3-C_q or 5-C_q); 139.53 (6-C_q); 147.78 (2-C_q or 4-C_q); 154.59 (2-C_q or 4-C_q); 192.52 (*C*HO). MS (70 eV, ES^+, *m/z* (%)): 227 ($M+H^+$). Anal. Calcd for $C_{10}H_{10}O_6$: C, 53.10%; H, 4.46%. Found: C, 52.91%; H, 4.33%.

2-Hydroxy-6-methoxy-4,5-methylenedioxybenzaldehyde **11**. Mp (°C): 121 (white powder). IR (KBr, cm^{-1}): 3435 (broad, OH); 1633 (C=O). ^1H NMR (270 MHz, $CDCl_3$): δ 4.12 (3H, s, OCH_3); 5.93 (2H, s, OCH_2O); 6.12 (1H, s, 3-C*H*); 10.03 (1H, s, C*H*O); 12.56 (1H, s, O*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 60.03 (O*C*H$_3$); 92.14 (3-*C*H); 101.60 (O*C*H$_2$O);

107.24 (1-C_q); 127.41 (C_q); 143.79 (C_q); 157.27 (C_q); 162.49 (C_q); 191.99 (*C*HO). MS (70 eV, ES^+, *m/z* (%)): 197 ($M+H^+$). Anal. Calcd for $C_9H_8O_5$: C, 55.11%; H, 4.11%. Found: C, 55.37%; H, 4.19%.

4.2.6. Ayapin (6,7-methylenedioxycoumarin) 1. *6H-[1,3]Dioxolo[4,5-g]chromen-6-one*. 2-Hydroxy-4,5-methylenedioxybenzaldehyde **6** (166 mg; 1 mmol) and methoxycarbonyltriphenylphosphorane (401 mg; 1.2 mmol) were dissolved in 5 ml of *N*,*N*-diethylaniline. The reaction mixture was stirred for 4 h at 220 °C under nitrogen atmosphere. The reaction mixture was cooled down to room temperature. The solvent was removed by vacuum distillation (0.01 mmHg/40–50 °C). After chromatography (50% diethyl ether, 50% hexane), 148 mg (78%) of pure ayapin **1** was obtained (white solid).

Mp (°C): 229–230 (lit. 231–232[26]). IR (KBr, cm^{-1}): 1705 (C=O), 1620, 1575. ^1H NMR (270 MHz, $CDCl_3$): δ 6.07 (2H, s, OCH_2O); 6.28 (1H, d, $J=9.6$ Hz, 3-C*H*); 6.82 and 6.83 (each 1H, each s, 5-C*H* and 8-C*H*); 7.58 (1H, d, $J=9.6$ Hz, 4-C*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 98.42 (8-*C*H); 102.35 (O*C*H_2O); 105.03 (5-*C*H); 112.68 (4a-C_q); 113.40 (3-*C*H);143.50 (4-*C*H); 144.92 (6-C_q); 151.25 (7- or 8a-C_q); 151.28 (7- or 8a-C_q); 161.26 (*C*=O). MS (70 eV, EI, *m/z* (%)): 191 (M+1, 14); 190 (M^+, 100); 162 (60); 161 (37); 81 (12); 79 (12); 76 (17); 53 (12); 51 (14). Anal. Calcd for $C_{10}H_6O_4$: C, 63.16%; H, 3.18%. Found: C, 63.36%; H, 3.28%.

4.2.7. 8-Methoxy-6,7-methylenedioxycoumarin 2. *4-Methoxy-6H-[1,3]dioxolo[4,5-g]chromen-6-one*. The synthesis of 8-methoxy-6,7-methylenedioxycoumarin **2** (169 mg; 0.77 mmol) from 2-hydroxy-3-methoxy-4,5-methylenedioxybenzaldehyde **9** (196 mg; 1.00 mmol) was analogous to the synthesis of ayapin **1**. Yield: 77% (white solid).

Mp (°C): 148–150 °C (lit. mp not reported[5]). IR (KBr, cm^{-1}): 1705 (C=O), 1585. ^1H NMR (270 MHz, $CDCl_3$): δ 4.12 (3H, s, OCH_3); 6.05 (2H, s, OCH_2O); 6.29 (1H, d, $J=9.6$ Hz, 3-C*H*); 6.57 (1H, s, 5-C*H*); 7.56 (1H, d, $J=9.6$ Hz, 4-C*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 60.70 (O*C*H_3); 99.33 (5-*C*H); 102.30 (O*C*H_2O); 113.30 (4a-C_q); 113.89 (3-*C*H); 131.91 (7- or 8a-C_q); 140.63 (7- or 8a-C_q); 143.47 (8-*C*H); 143.66 (4-*C*H); 145.60 (6-C_q); 160.59 (*C*=O). MS (70 eV, EI, *m/z* (%)): 220 (M^+, 100); 192 (51); 190 (32); 162 (22); 147 (22); 79 (26); 63 (21); 53 (19); 51 (29); 44 (27). Anal. Calcd for $C_{11}H_8O_5$: C, 60.01%; H, 3.66%. Found: C, 59.95%; H, 3.48%.

4.2.8. 5-Methoxy-6,7-methylenedioxycoumarin 3. *9-Methoxy-6H-[1,3]dioxolo[4,5-g]chromen-6-one*. The synthesis of 5-methoxy-6,7-methylenedioxycoumarin **3** (8.7 mg; 0.04 mmol) from 2-hydroxy-6-methoxy-4,5-methylenedioxybenzaldehyde **11** (9.8 mg; 0.05 mmol) was analogous to the synthesis of ayapin **1**. Yield: 79% (white solid).

Mp (°C): 192 (lit. 200–202,[6] 192–194[7]). IR (KBr, cm^{-1}): 1740 (C=O), 1627 (C=C). ^1H NMR (270 MHz, $CDCl_3$): δ 4.14 (3H, s, OCH_3); 6.01 (2H, s, OCH_2O); 6.21 (1H, d, $J=9.9$ Hz, 3-C*H*); 6.54 (1H, s, 8-C*H*); 7.95 (1H, d, $J=9.9$ Hz, 4-C*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 59.99 (*C*H_3O); 92.50 (8-*C*H); 101.83 (O*C*H_2O); 106.63 (4a-C_q); 111.74 (3-*C*H); 131.76 (6-C_q); 138.05 (5-C_q); 138.84 (4-*C*H); 151.56 (7- or 8a-C_q); 152.67 (7- or 8a-C_q); 161.33 (*C*=O). MS (70 eV, ES^+, *m/z* (%)): 221 ($M+H^+$). Anal. Calcd for $C_{11}H_8O_5$: C, 60.01%; H, 3.66%. Found: C, 60.23%; H, 3.51%.

4.2.9. 5,8-Dimethoxy-6,7-methylenedioxycoumarin 4. *4,9-Dimethoxy-6H-[1,3]dioxolo[4,5-g]chromen-6-one*. The synthesis of 5,8-dimethoxy-6,7-methylenedioxycoumarin **4** (41 mg; 0.16 mmol) out of 2-hydroxy-3,5-dimethoxy-4,5-methylenedioxybenzaldehyde **9** (45.2 mg; 0.2 mmol) was analogous to the synthesis of ayapin **1**. Yield: 82% (yellow solid).

Mp (°C): 131–133 (lit. mp not reported[32]). IR (KBr, cm^{-1}): 1724 (C=O), 1620, 1590. ^1H NMR (270 MHz, $CDCl_3$): δ 4.02 (3H, s, 5-OCH_3); 4.06 (3H, s, 8-OCH_3) 6.03 (2H, s, OCH_2O); 6.22 (1H, d, $J=9.7$ Hz, 3-C*H*); 7.93 (1H, d, $J=9.7$ Hz, 4-C*H*). ^{13}C NMR (68 MHz, $CDCl_3$): δ 60.22 (8-O*C*H_3); 61.25 (5-O*C*H_3); 102.21 (O*C*H_2O); 107.10 (4a-C_q); 112.24 (3-*C*H); 127.04 (C_q); 132.92 (C_q); 133.48 (C_q); 138.92 (4-*C*H); 143.07 (7- or 8a-C_q); 143.83 (7- or 8a-C_q); 160.59 (*C*=O). MS (70 eV, ES^+, *m/z* (%)): 251 ($M+H^+$). Anal. Calcd for $C_{12}H_{10}O_6$: C, 57.60%; H, 4.03%. Found: C, 57.50%; H, 4.20%.

Acknowledgements

This work was financially supported by the Special Research Fund (BOF) of Ghent University, the Fund for Scientific Research-Flanders (FWO) and SECyT (Argentina).

References and notes

1. Debenedetti, S. L.; Nadinic, E. L.; Coussio, J. D.; De Kimpe, N.; Feneau-Dupont, J.; Declercq, J.-P. *Phytochemistry* **1991**, 2757–2758.
2. Debenedetti, S. L.; Nadinic, E. L.; Gomez, M. A.; Coussio, J. D.; De Kimpe, N.; Boeykens, M. *Phytochemistry* **1992**, 3284–3285.
3. Debenedetti, S. L.; Nadinic, E. L.; Gomez, M. A.; Coussio, J. D.; De Kimpe, N.; Boeykens, M. *Phytochemistry* **1996**, 563–564.
4. Debenedetti, S. L.; Abbaspour Tehrani, K.; Van Puyvelde, L.; De Kimpe, N. *Phytochemistry* **1999**, 701–703.
5. Sarker, S. D.; Gray, A. I.; Waterman, P. G.; Armstrong, J. A. *J. Nat. Prod.* **1994**, 1549–1551.
6. Maldonado, E.; Hernandez, E.; Ortega, A. *Phytochemistry* **1992**, 1413–1414.
7. Debenedetti, S. L.; Palacios, P. S.; Nadinic, E. L.; Coussio, J. D.; De Kimpe, N.; Boeykens, M.; Feneau-Dupont, J.; Declercq, J.-P. *J. Nat. Prod.* **1994**, 1539–1542.
8. Bose, P. K.; Roy, A. C. *J. Indian Chem. Soc.* **1936**, *13*, 586. *Chem. Abstr.* **1937**, *31*, 1787.
9. Gutierrez, M. C.; Parry, A.; Tena, M.; Jorrin, J.; Edwards, R. *Phytochemistry* **1995**, 1185–1191.

10. Hiroko, S.; Sashida, Y.; Ohshima, Y. *Phytochemistry* **1979**, 1761–1762.
11. Debenedetti, S. L.; De Kimpe, N.; Boeykens, M.; Coussio, J. D.; Kesteleyn, B. *Phytochemistry* **1997**, 1515–1517.
12. Palacios, P. S.; Rojo, A. A.; Coussio, J. D.; De Kimpe, N.; Debenedetti, S. L. *Planta Med.* **1999**, *65*, 294–295.
13. Ishii, H.; Kaneko, Y.; Miyazaki, H.; Harayama, T. *Chem. Pharm. Bull.* **1991**, 3100–3102.
14. Ishii, H.; Kenmotsu, K.; Döpke, W.; Harayama, T. *Chem. Pharm. Bull.* **1992**, 1770–1772.
15. Ishii, H.; Ishikawa, T.; Wada, H.; Miyazaki, H.; Kaneko, Y.; Harayama, T. *Chem. Pharm. Bull.* **1992**, 2614–2619.
16. Harayama, T.; Nakatsuka, K.; Katsuno, K.; Nishioka, H.; Murakami, K.; Fujii, M.; Hayashida, N.; Takeuchi, Y. *Chem. Express* **1993**, *8*, 245–248.
17. Gattermann, L.; Köbner, M. *Ber. Dtsch. Chem. Ges.* **1899**, *32*, 278–283.
18. Fukui, K.; Nakayama, M. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 1321–1323.
19. Dreyer, D. L. *J. Org. Chem.* **1957**, *33*, 1112–1115.
20. Bacon, R. G. R.; Rennison, S. C. *J. Chem. Soc. (C)* **1969**, 312–315.
21. Keegstra, M. A.; Peters, T. H. A.; Brandsma, L. *Tetrahedron* **1992**, *48*, 3633–3652.
22. Capdevielle, P.; Maumy, M. *Tetrahedron Lett.* **1993**, *34*, 1007–1010.
23. Besson, T.; Ruiz, N.; Coudert, G.; Guillaumet, G. *Tetrahedron* **1995**, *51*, 3197–3204.
24. Royer, R.; René, L.; Cavier, R.; Lemoine, J. *Eur. J. Med. Chem.* **1977**, *12*, 455–458.
25. Demyttenaere, J.; Van Syngel, K.; Markusse, A. P.; Vervisch, S.; Debenedetti, S. L.; De Kimpe, N. *Tetrahedron* **2002**, *58*, 2163–2166.
26. Castillo, P.; Rodriguez-Ubis, J. C.; Rodriguez, F. *Synthesis* **1986**, 839–840.
27. Riviero, E.; Fernandez, N.; Monczor, F.; Debenedetti, S. L.; Rossi, J.; Baldi, A.; Shayo, C.; Davio, C. *Medicina* **2002**, *62*, 512.
28. Riviero, M. E.; Shayo, C.; Monczor, F.; Fernandez, N.; Baldi, A.; De Kimpe, N.; Rossi, J.; Debenedetti, S. L.; Davio, C. *Cancer Lett.* **2004**, *210*, 178–189.
29. Estevez, R.; Gonzalez, A. G. *Phytochemistry* **1970**, 833–840.
30. Gonzalez, A. G.; Breton, J. L.; Lopez Dorta, H.; Martinez Iniguez, M. A.; Rodriguez Luis, F. *An. Quim.* **1973**, *69*, 1013–1029.
31. Wu, T.-S.; Tsang, Z.-J.; Wu, P.-L.; Lin, F.-W.; Li, C.-Y.; Teng, C.-M.; Lee, K.-H. *Bioorg. Med. Chem.* **2001**, *9*, 77–83.
32. Baetas, A. C. S.; Arruda, M. S. P.; Müller, A. H.; Arruda, A. C. *J. Braz. Chem. Soc.* **1999**, *10*, 181–183.