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Homoisoflavonoids from *Ophiopogon japonicus* Ker-Gawler

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Dedicated to Professor G. Adam on the occasion of his 70th birthday.

Abstract

From the ethyl acetate extract of the tuberous roots of *Ophiopogon japonicus* (Liliaceae) eight known and five new homoisoflavonoidal compounds were isolated. The new compounds are 5,7-dihydroxy-8-methoxy-6-methyl-3-(2′-hydroxy-4′-methoxybenzyl)chroman-4-one (**1**), 7-hydroxy-5,8-dimethoxy-6-methyl-3-(2′-hydroxy-4′-methoxybenzyl)chroman-4-one (**2**), 5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxy-3′-methoxybenzyl)chroman-4-one (**3**), 2,5,7-trihydroxy-6,8-dimethyl-3-(3′,4′-methylenedioxybenzyl)-chroman-4-one (**4**) and 2,5,7-trihydroxy-6,8-dimethyl-3-(4′-methoxybenzyl)chroman-4-one (**5**). Their structures have been elucidated by mass and NMR spectroscopy. Compounds **4** and **5** are the first isolated homoisoflavonoids with a hemiacetal function at position 2.

Keywords: O*phiopogon japonicus*; Liliaceae; Homoisoflavonoids

1. Introduction

Ophiopogon japonicus Ker-Gawler (Liliaceae) is an evergreen perennial, widely used in traditional Chinese medicine in combination with *Panax ginseng* and *Schisandra chinensis* for its pharmacological effects on the cardiovascular system (Adinolfi et al., 1990; Zhu et al., 1987). In the folk medicine of Viet Nam it serves as expectorant, anti-cough and tonic agent (Do, 1991). From the subterranean part of *O. japonicus* many steroidal glycosides (Watanabe et al., 1977; Asano et al., 1993) and homoisoflavonoidal compounds (Tada et al., 1980; Kaneda et al., 1983) as well as monoterpene glycosides (Adinolfi et al., 1990; Kaneda et al., 1983) have been reported previously. In this paper we describe the isolation and the structural elucidation of five new homoisoflavonoids from the ethyl acetate extract of the roots of *O. japonicus*. Homoisoflavonoids have been shown to possesss antiinflammatory, antiallergic, antihistaminic and angioprotective activities and have been detected as potent phosphodiesterase inhibitors (Amschler et al., 1996).

2. Results and discussion

The tuberous roots of *O. japonicus* were extracted with hot ethanol. The ethanol extract has been treated further by extraction with *n*-hexane, ethyl acetate and *n*-butanol, successively. After chromatographic separation of the ethyl acetate extract 13 homoisoflavonoids were isolated. Besides five new compounds (**1–5**), eight known homoisoflavonoidal compounds have also been obtained. The known compounds **6–13** were identified as 5,7-trihydroxy-6,8-dimethyl-3-(2′-hydroxy-3′,4′-methylenedioxybenzyl)chromone (**6**), methylophiopogonone A (**7**), ophiopogonanone A (**8**), methylophiopogonanone B (**9**), methylophiopogonanone A (**10**), 6-formyl-isoophiopoganone A (**11**), 5-hydroxy-7,8-dimethoxy-6-methyl-3-(3′,4′-dihydroxybenzyl)chroman-4-one (**12**) and 5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxy-3′8′-dimethoxybenzyl)chroman-4-one (**13**) based on the comparison of their spectroscopic data with those described in the

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literature (Tada et al., 1980; Kaneda et al., 1983; Asano et al., 1993; Watanabe et al., 1985).

Of the new derivatives, compound **1** was isolated as amorphous powder. The ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H-correlation (COSY) spectra revealed the presence of a homoisoflavonoid skeleton substituted with one aromatic methyl group, two methoxy groups, one chelated hydroxy group and two further hydroxy functions. The EIMS spectrum showed the molecular peak at m/z 360 $[M]^{+}$ and two fragment peaks due to the B-ring at m/z 164 and 137 and a fragment peak at m/z 197 derived from a retro-Diels–Alder reaction. The elemental composition of compound **1** was $C_{19}H_{20}O_7$ according to high-resolution mass spectrometry. Based on these MS and NMR data, this compound had one methyl, one methoxy group and two hydroxy groups in

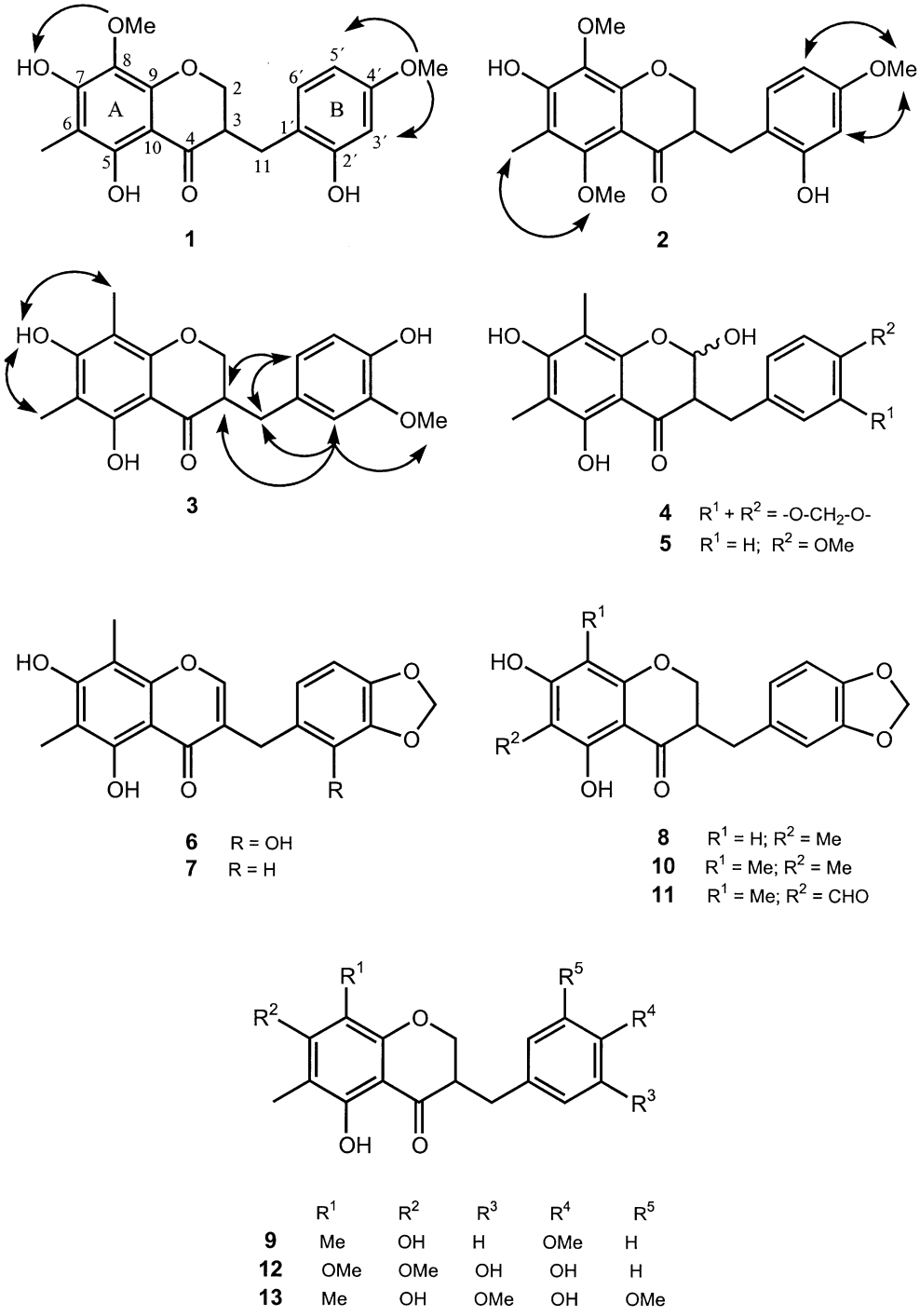

Fig. 1. Constituents from *Ophiopogon japonicus* (important NOE correlations are indicated as arrows).

the A-ring as well as one hydroxy group and one methoxy group in the B-ring. The location of the functional groups and the assignment of the NMR signals were achieved by analysis of the direct and long-range ^{1}H-^{13}C-correlations (Table 3) as well as of the NOE-difference spectra (Fig. 1). These data led to the structure of 5,7-dihydroxy-8-methoxy-6-methyl-3-(2′-hydroxy-4′-methoxybenzyl)chroman-4-one for compound **1**.

Compound **2** showed the molecular peak at *m*/*z* 374 $[M]^+$ and the base peak at *m*/*z* 137 ($C_8H_9O_2$) due to the B-ring moiety. The elemental composition of compound **2** was $C_{20}H_{22}O_7$ according to high-resolution mass spectrometry. A comparison of ^{1}H NMR and EIMS spectra of compounds **1** and **2** suggested that both compounds possess the same B-ring structure of 2′-hydroxy-4′-methoxybenzyl. Also, the ^{1}H and ^{13}C NMR spectra were very similar, but exhibited one methoxy signal more (δ 3.76 and 61.3) and a chelated hydroxy signal less in **2** indicating a methoxy substituent at positon C-5. This suggestion was confirmed by correlations in the NOESY (Fig. 1) and HMBC (Table 3) experiments. All spectroscopic data led to the structure 7-hydroxy-5,8-dimethoxy-6-methyl-3-(2′-hydroxy-4′-methoxybenzyl)-chroman-4-one for **2**.

Compound **3** was isolated as amorphous powder. The EIMS spectrum showed the molecular peak at *m*/*z* 344 $[M]^+$ and the base peak at *m*/*z* 137 due to the B-ring moiety. The elemental composition of compound **3** was obtained as $C_{19}H_{20}O_6$ from high-resolution ESIMS. The proton signals and the MS-fragmentations revealed the presence of two aromatic methyl groups and two hydroxy groups in the A-ring and a methoxy group and a hydroxy group in the B-ring. The presence of one chelated hydroxy group at δ 12.38 suggested that it was located at C-5. The two methyl groups in the A-ring were connected at C-6 and C-8, because interactions between these methyl signals and the signal of 7-OH were observed in the NOESY spectrum. This was supported by the recorded HMBC correlations (Table 3). The location of the methoxy group in the B-ring at

Table 1
^{1}H-NMR data of compounds **1**–**5** measured in $CDCl_3$ [300 MHz, δ, multiplicity, ^{1}H–^{1}H-coupling constants (Hz) in parentheses]

	1	**2**	**3**	**4**[a]		**5**	
H				**4A**[b]	**4B**	**5A**[b]	**5B**
2A	4.55 *dd* (11.1/5.0)	4.61 *dd* (11.1/5.3)	4.29 *dd* (11.1/3.8)	5.32 *dd* (4.3/3.4)	5.28 *dd* (4.5/3.3)	5.42 *d* (2.9)	5.48 *d* (3.2)
2B	4.22 *dd* (11.1/10.8)	4.21 *dd* (12.0/11.1)	4.12 *dd* (11.1/6.9)	–	–	–	–
3	3.10 *m*	3.05 *m*	2.79 *m*	3.00 *dddd* (10.8/4.7/3.4/1.6)	2.78 *ddd* (9.1/5.1/3.3)	3.15 *ddd* (3.2/4.7/10.8)	2.99 *m*
2-OH	–	–	–	2.77 *dd* (4.3/1.6)	2.91 *d* (4.5)	n.d.	n.d.
5-OH	11.91 *s*	–	12.38 *s*	12.22 *s*	12.31 *s*	12.20 *s*	12.28 *s*
5-OMe	–	3.76 *s*	–	–	–	–	–
6-CH$_3$	2.04 *s*	2.09 *s*	2.03 *s*	2.01[d] *s*	2.01[d] *s*	2.00[c] *s*	2.06[c] *s*
7-OH	6.62 *s*	n.d.	5.43 *br s*	5.27 *s*	5.32	5.55 *br s*	5.55 *br s*
8-CH$_3$	–	–	2.07 *s*	1.97 *s*	2.02[d] *s*	2.06[c] *s*	2.07[c] *s*
8-OMe	3.85 *s*	3.87 *s*	–	–	–	–	–
11A	2.99 *dd* (14.7/5.3)	2.91 *d* (5.0)	3.16 *dd* (13.5/3.8)	3.39 *dd* (14.2/4.7)	2.92 *dd* (13.5/5.1)	3.46 *dd* (4.5/14.2)	2.90 *d* (12.0)
11B	2.89 *dd* (14.7/5.9)	2.91 *d* (5.0)	2.69 *dd* (13.5/10.3)	2.66 *dd* (14.2/10.8)	2.84 *dd* (13.5/9.1)	2.75 *dd* (14.2/10.7)	2.90 *d* (12.0)
2′	–	–	6.74 *d* (1.8)	6.68 *d* (1.7)	6.72 *d* (1.7)	7.18 *d* (8.8)	7.18 *d* (8.8)
3′	6.46 *d* (2.6)	6.49 *d* (2.6)	–	–	–	6.86 *d* (8.8)	6.85 *d* (8.8)
5′	6.42 *dd* (8.2/2.6)	6.39 *dd* (8.3/2.6)	6.86 *d* (7.9)	6.72 *d* (7.9)	6.70 *d* (7.9)	6.86 *d* (8.8)	6.85 *d* (8.8)
6′	6.93 *d* (8.2)	6.90 *d* (8.3)	6.72 *dd* (7.9/1.8)	6.63 *dd* (7.9/1.7)	6.66 *dd* (7.9/1.7)	7.18 *d* (8.8)	7.18 *d* (8.8)
3′-OMe	–	–	3.88 *s*	–	–	–	–
4′-OMe	3.76 *s*	3.75 *s*		–	–	3.80 *s*	3.79 *s*
O-CH$_2$-O	–	–	–	5.85 *d* (1.4) 5.84 *d* (1.4)	5.83 *d* (1.4) 5.82 *d* (1.4)	–	–

[a] Measured in $CDCl_3$:C_6D_6 v/v 85:15 (500 MHz), chemical shifts in $CDCl_3$ see Experimental.
[b] The main component in the mixture.
[c] May be exchanged in each column.
[d] May be exchanged between **4A** and **4B**.

position 3′ was determined by the long-range-correlation of the methoxy protons with H-2′ (6.74, *d*, $J = 1.8$ Hz) and the NOE effects of H-2′ with 3′-OMe. These data defined **3** as 5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxy-3′-methoxybenzyl)chroman-4-one.

The observed coupling constants between H-2 and H-3 indicated an axial orientation of H-3 in compounds **1** ($J = 10.8/5.0$ Hz) and **2** ($J = 12.0/5.3$ Hz) and an equatorial orientation in compound **3** ($J = 6.9/3.8$ Hz). The orientation of H-3 depends from the configuration at C-3 and from the conformation of the C-ring. Several homoisoflavons were shown to possess the *R*-configuration at C-3 irrespective of the orientation of H-3 (Adinolfi et al., 1988).

The ^{1}H NMR spectrum of compound **4** showed two sets of signals with mostly identical coupling patterns but with different chemical shifts, supporting the assumption, that compound **4** existed as a mixture of two isomers in the ratio of about 1:0.87 (**4A**:**4B**) which could not be separated due to the similarity of both compounds. The EIMS spectrum of **4** exhibited the molecular peak at *m*/*z* 358 and the base peak at *m*/*z* 135 derived from the B-ring moiety and two fragment peaks from the A-ring at *m*/*z* 181 and *m*/*z* 223. High-resolution mass spectrometry indicated an elemental composition of $C_{19}H_{18}O_7$ for **4**. The comparison of ^{1}H NMR spectra of compounds **1** and **4** suggested that **4** also possessed a homoisoflavonoid skeleton. But instead of the oxy-methylene proton signals of H-2 in compound **1**, one hydroxylated methin signal appeared. Based on the fragmentation in the MS spectrum and the proton signals it was concluded that a hydroxy group is connected to C-2, resulting in a hemiacetal function. This function is the reason that the compound formed two isomers. In addition, this result was supported by the ^{1}H–^{1}H-COSY, which showed the correlations for H-3/H-2, H-11/H-3 and 2-OH/H-2. Furthermore, compound **4** displayed signals for a methylenedioxygroup with HMBC couplings between their protons and C-3′ and C-4′. Consequently, the structure of **4** was determined as 2,5,7-trihydroxy-6,8-dimethyl-3-(3′,4′-methylenedioxybenzyl)chroman-4-one.

The EIMS spectra of compound **5** showed the molecular peak at *m*/*z* 344 $[M]^+$ and the base peak at *m*/*z* 121. The elemental composition of **5** was $C_{19}H_{20}O_6$ according to high-resolution mass spectrometry. From the ^{1}H NMR spectra it was concluded to be a mixture of two isomers in the ratio of about 1:0.79 (**5A**:**5B**). The ^{1}H NMR spectra of **4** and **5** were very similar. The signals for the ring A corresponded to each other, but the ring B was a *para* disubstitued benzene ring (δ 6.86 and 7.18, $J = 8.8$ Hz) with a methoxy substituent (δ 3.80/

Table 2
^{13}C NMR data of compounds **1–5** measured in $CDCl_3$ (76 MHz, δ)

	1	**2**	**3**	**4**		**5**	
C				**4A**	**4B**	**5A**	**5B**
2	70.3	70.5	68.9	95.0	96.0	95.1	96.1
3	45.9	47.6	46.9	51.1	52.6	51.2	52.7
4	199.1	193.9	198.2	195.7	196.7	195.9	196.9
5	157.8	155.8	159.4	158.7	159.2	158.7	159.2
6	104.3	112.6	102.8	103.1	103.3	103.1[a]	103.3[a]
7	156.4	154.5	160.5	160.4	160.8	160.8	160.4
8	126.8	130.5	101.5	102.0	102.4	102.1[a]	101.6[a]
9	150.6	153.2	157.6	153.2	152.9	153.3	152.9
10	101.9	108.6	102.2	102.0	101.6	102.1	102.1
11	26.1	26.0	32.7	29.9	34.3	29.2	29.8
6-CH$_3$	7.1	8.0	7.5	7.0	7.0	7.6[b]	7.7[b]
8-CH$_3$	–	–	7.0	7.6	7.7	7.0[b]	7.0[b]
3′-OMe	–	–	55.9	–	–	–	–
4′-OMe	55.3	55.3	–	–	–	55.3	55.3
8-OMe	61.5	61.3	–	–	–	–	–
5-OMe	–	61.3	–	–	–	–	–
1′	116.1	116.5	129.8	131.9	131.0	129.4	129.4
2′	155.2	155.6	114.3[a]	109.3	109.5	129.9	130.1
3′	102.6	102.7	146.5	147.6	147.6	114.0	113.9
4′	159.8	159.8	144.2	146.0	146.3	158.1	158.3
5′	106.4	106.3	111.3[a]	108.4	108.3	114.0	113.9
6′	131.5	131.4	121.8[a]	121.8	122.3	129.9	130.1
O–CH$_2$–O	–	–	–	100.9	100.9	–	–

[a] May be exchanged in each column.
[b] May be exchanged in each column.

Table 3
Important HMBC correlations of compounds **1–5**

H	1	2	3	4	5
5-OH	C-5, C-6, C-10			C-5, C-6, C-10	C-5, C-6, C-10
5-OMe		C-5			
6-CH_3	C-6, C-5, C-7, C-8	C-6, C-5, C-7	C-6, C-5, C-7	C-6, C-5, C-7	C-6, C-7
7-OH	C-7, C-6, C-8			C-7, C-6, C-8, C-9	
8-CH_3			C-8, C-7, C-9	C-8, C-7, C-9	C-8, C-7, C-9
8-OMe	C-8	C-8			
11	C-3, C-1′, C-2, C-4, C-2′, C-6′	C-3, C-1′, C-2, C-4, C-2′, C-6′		C-3, C-1′, C-2, C-4, C-2′, C-6′	C-3, C-1′, C-2, C-4
3′-OMe			C-3′		
4′-OMe	C-4′	C-4′			C-4′
O-CH_2-O				C-3′, C-4′	

3.79) in **5**. Thus, **5** was supposed to be 2,5,7-trihydroxy-6,8-dimethyl-3-(4′-methoxybenzyl)chroman-4-one. This was confirmed by analysis of the COSY, HSQC and HMBC experiments.

The correlations found in the ^{1}H–^{1}H-COSY, HSQC, HMBC and the results of the 1D-NOE experiments allowed a complete assignment of all ^{1}H and ^{13}C NMR signals for **1–5** (Tables 1 and 2).

3. Experimental

3.1. Apparatus and chemicals

EIMS: AMD 402, 70 eV. HR-ESI-MS: BRUKER BIOAPEX 70e Fourier transform ion cyclotron resonance mass spectrometer equipped with an Infinity^{TM} cell, a 7.0 Tesla supraconducting magnet, a RF-only hexapole ion guide and an external electrospray ion source (Apollo^{TM}). NMR: VARIAN MERCURY 300, INOVA 500, internal reference TMS = 0 ppm (^{1}H and 2D NMR); $CDCl_3$ = 77 ppm (^{13}C). $[\alpha]_D$: JASCO Digital Polarimeter Ver. 100.19, CC: silica gel 60, 40–60 μm (Merck), Sephadex LH-20 (Fluka). Prep. TLC: precoated plates, silica gel 60, F_{254}, thickness 0.5 mm (Merck).

3.2. Plant material, extraction and isolation

The roots of *O. japonicus* Ker-Gawler were bought in the market for medicinal plants in Hanoi, July 2000. A voucher specimen was deposited in the herbarium of the Institute of Chemistry, Hanoi with No. 3. The roots were dried at 40 °C, ground and extracted exhaustively with hot ethanol (95%). The organic solvent was evaporated in vacuo and 800 g of the residue were suspended in 1000 ml water and extracted with *n*-hexane, EtOAc and *n*-BuOH, successively. The solvents were evaporated in vacuo to afford the *n*-hexane (4 g), EtOAc (5.2 g) and *n*-BuOH (34 g) extracts.

The EtOAc extract was chromatographed over a Sephadex LH-20 column, eluting with methanol to afford 10 fractions which were combined according to TLC monitoring. Compound **11** (5.5 mg) was isolated from the first fraction by CC on silica gel (*n*-hexane:$CHCl_3$ = 7:3) followed by prep. TLC developed with $CHCl_3$. Further separation of fraction 3 by repeated CC on silica gel using $CHCl_3$ and on Sephadex LH-20 using EtOH gave the compounds **9** (4.5 mg) and **10** (5.0 mg).

The 4th fraction was further purified over a flash silica gel column, eluting with *n*-hexane:$CHCl_3$ (7:3) and then with $CHCl_3$ to yield **1** (7.9 mg) as amorphous powder, $[\alpha]_D^{22}$ −12.6° ($CHCl_3$:MeOH = 1:2, *c* 0.29). EIMS *m/z* (rel.int): 360 $[M]^+$, 342 (35), 327 (12.2), 236 (11.4), 224 (41.4), 209 (21.4), 197 (2.8), 181 (5), 163 (5), 137 (100). HR-ESI-MS: *m/z* 361.12756 $[M+H]^+$ (calc. for $C_{19}H_{21}O_7$ 361.12818). ^{1}H and ^{13}C NMR data: see Tables 1 and 2.

The 5th fraction was chromatographed over a flash silica gel column, eluting with $CHCl_3$ to afford 5 fractions. The fraction containing compound **2** was further purified by flash silica gel column, eluting with $CHCl_3$:MeOH = 97:3, followed by prep. TLC developing with $CHCl_3$:MeOH (95:5) to yield **2** (11.0 mg), $[\alpha]_D^{22} = -7.74°$ (MeOH, *c* 0.97). EIMS *m/z* (rel. int): 374 $[M]^+$, 356 (73.3), 341 (31.6), 237 (71.6), 223 (26.7), HR-ESI-MS: *m/z* 361.12756 $[M+Na]^+$ (calc. for $C_{17}H_{22}O_7Na$ 361.12577). 181 (28.3), 162 (30), 151 (33.3), 137 (100). HR-ESI-MS: *m/z* 397.12480 $[M+Na]^+$ (calc. for $C_{20}H_{22}O_7Na$ 397.12577). ^{1}H and ^{13}C NMR: see Tables 1 and 2.

The fraction eluted from silica gel previous to compound **2** was separated by CC on RP18 eluting with MeOH:H_20 (8:2) and by prep. TLC developing in *n*-hexane:EtOAc (1:1) to yield compound **13** (2 mg).

The fraction containing compound **3** from the 5th fraction was given over a flash silica gel column, eluting with $CHCl_3$:MeOH = 95:5 to obtain compound **3** (3 mg), EIMS *m/z* (rel.int): 344 $[M]^+$, 326 (1.4), 208 (100), 181 (14.3), 137 (90.7). HR-ESI-MS: *m/z* 367.11509

$[M+Na]^+$ (calc. for $C_{19}H_{20}O_6Na$ 367.11521). ^1H and ^{13}C NMR: see Tables 1 and 2.

The 6th fraction was given over a flash silica gel column, washing with $CHCl_3$:MeOH (95:5), followed by prep.TLC developing with $CHCl_3$:MeOH (93:7) to give a mixture of compound **4** and **5**. Compound **4** and **5** were separated from this mixture by using sephadex LH-20, eluting with ethanol.

Compound **4** (6.5 mg) was obtained as amorphous powder, $[\alpha]_D^{22} = 0.5^\circ$ (MeOH, *c* 0.5). EIMS *m*/*z* (rel.int): 358 $[M]^+$, 340 (35.7), 329 (8.5), 223 (3.5), 218 (12.1), 181 (18.5), 160 (12.1), 135 (100). HR-ESI-MS: *m*/*z* 359.11214 $[M+H]^+$ (calc. for $C_{19}H_{19}O_7$ 359.11253). ^1H NMR ($CDCl_3$) **4A**: 1.99 *s* (CH_3-6*), 2.06 *s* (CH_3-8*), 2.72 *dd* (H-11), 3.43 *dd*, 4.7, 14.1 (H-11), 2.8–3.3 *m* (H-3), 5.49 *s* (7-OH), 5.94 *s* (O–CH_2O), 6.6–6.8 *m* (H-2′, H-5′, H-6′), 12.25 *s* (5-OH).

4B: 2.06 *s* (CH_3-6*), 2.07 *s* (CH_3-8*), 2.8-3.3 *m* (H-3, 2H-11), 5.44 *s* (7-OH), 5.93 *s* (O–CH_2O–), 6.6–6.8 *m* (H-2′, H-5′, H-6′), 12.25 *s* (5-OH). (*may be exchanged in the compound)

Compound **5** (2.6 mg) was isolated as amorphous powder, $[\alpha]_D^{22} = -8^\circ$ (MeOH, *c* 0.22). EIMS *m*/*z* (rel. int) 344 $[M]^+$, 315 (5), 236 (4.3), 223 (4.6), 208 (4.3), 181 (19.3), 164 (4.3), 121 (100). HR-ESI-MS: *m*/*z* 345.13294 $[M+H]^+$ (calc. for $C_{19}H_{21}O_6$ 345.13326). ^1H and ^{13}C NMR: see Tables 1 and 2.

Rechromatography of fraction 7 by CC on silica gel ($CHCl_3$) followed by prep. TLC ($CHCl_3$:MeOH = 93:7 and *n*-hexane-EtOAc = 1:1) resulted in the isolation of the compounds **6** (4.0 mg), **7** (0.8 mg) and **8** (3.6 mg). Compound **12** (2.0 mg) was purified from the same fraction by CC on silica gel ($CHCl_3$), prep. TLC ($CHCl_3$:MeOH = 93:7) and CC on RP18 (MeOH:H_2O = 8:2).

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