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Polar constituents of celery seed

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Abstract

From the water-soluble portion of the methanol extract of celery seed (fruit of *Apium graveolens* L.) five sesquiterpenoid glucosides (celerioside A–E) and three phthalide glycosides (celephtalide A–C) were isolated together with six aromatic compound glucosides, two norcarotenoid glucosides and a lignan glucoside. Their structures were determined by spectral investigations.

Keywords: Celery seed; *Apium graveolens* L. fruit; Umbelliferae; Sesquiterpenoid glucoside; Celerioside; Phthalide glycoside; Celephtalide

1. Introduction

Celery (*Apium graveolens* L.; Umbelliferae) has been cultivated as a common European plant since antiquity and used as a popular aromatic herb and spice. Its fruit, celery seed, has been used for medicinal purposes as a diuretic for bladder and kidney complaints, and an adjuvant in arthritic and rheumatic conditions (Wichtl, 1994). Celery seed tea is said to promote rest and sleep (Norman, 1990). As the constituents of this fruit, an essential oil (2–3%) rich in limonene (main; 60%) and selinene (10%) (Fehr, 1979), furocoumarins (Garg et al., 1979) and glucosides of furocoumarin and flavonoid (Garg et al., 1980) have been reported. However, no terpenoid glycoside components have been reported from the water-soluble portion of this fruit. In continuation of our studies on the water-soluble constituents of spices (Fujimatu et al., 2003), we undertook an investigation of celery seed.

2. Results and discussion

Commercial celery seed was extracted with 70% aqueous methanol, and the methanolic extract was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was subjected to Amberlite XAD-II chromatography to give water and methanol eluate fractions. The methanol eluate fraction was further purified on Sephadex LH-20, and subjected to a combination of silica gel, Lobar RP-8 column chromatography and HPLC to isolate five sesquiterpenoid glucosides (**1**–**5**), two norcarotenoid glucosides (**6** and **7**), three phthalide glycosides (**8**–**10**), six aromatic compound glycosides (**11**–**16**), and a lignan glucoside (**17**). Among them, **1**–**5**, and **8**–**10** are new compounds. All new glucosides described in this paper were β-D-glucopyranosides as shown by their ^{1}H and ^{13}C NMR spectroscopic data (Tables 1–4), and this was confirmed by hydrolysis to yield D-glucose and/or comparison of the $[M]_D$ values with those of their aglycones (Klyne, 1950) except **4**. Their molecular formulae were suggested from the accurate mass number of the $[M+H]^+$ or $[M+Na]^+$ ion peak in the high-resolution positive FAB-MS.

Celeroside A (**1**; $C_{21}H_{34}O_9$, mp 223–225 °C, $[\alpha]_D^{23}$ $+19°$) showed $[M+Na]^+$, $[M+H]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 453, 431 and 251 in the positive-ion FAB-MS, and an $[M-H]^-$ ion peak at *m*/*z* 429 in the negative-ion FAB-MS. The NMR spectral data (Tables 1 and 2) of **1** established the presence of one β-glucopyranosyl group, and three *tert*-methyls, five methylenes, three methine (one of them was oxygenated), three quaternary carbons (two of them were oxygenated), and one carbonyl group (Table 1 and 2). Enzymatic hydrolysis of **1** with β-glucosidase gave an aglycone (**1a**; $C_{15}H_{24}O_4$, an amorphous powder, $[\alpha]_D^{23}$ $+30°$) and D-glucose, and it was suggested to be a

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Table 1
H NMR chemical shifts of **1**–**5** (in pyridine-d_5, 500 MHz)[a]

	1	**2**	**3**		**4**	**5**
H-1*ax*	3.90 1H, *dd* (6.0, 10.5)	3.74 1H, *dd* (8.0, 8.0)	3.78 1H, *dd* (3.5, 9.5)	H-1*ax*	3.54 1H, *d* (9.5)	3.66 1H, *dd* (3.5, 9.5)
H-2*ax*	1.98 1H, *dddd* (6.0, 10.5, 13.0, 13.0)	2.14 1H, *dddd* (5.0, 8.0, 13.0, 13.0)	2.17 1H, *dddd* (3.0, 9.5, 13.5, 13.5)	H-2*ax*	4.08 1H, *ddd* (5.5, 9.5, 11.0)	–
eq	2.22 1H, *br ddd* (6.0, 6.0, 13.0)	2.14 1H, *m*	2.15 1H, *br d* (13.5)	H_2-2	–	1.95 2H, *m*
H-3*ax*	1.66 1H, *ddd* (6.0, 13.0, 13.0)	1.41 1H, *ddd* (5.0, 13.0, 13.0)	2.13 1H, *ddd* (4.5, 13.5, 13.5)	H-3*ax*	2.47 1H, *dd* (11.0, 13.0)	1.93 1H, *m*
eq	1.81 1H, *br dd* (6.0, 13.0)	1.54 1H, *br d* (13.0)	2.35 1H, *ddd* (3.0, 3.0, 13.5)	*eq*	2.91 1H, *dd* (5.5, 13.0)	1.99 1H, *ddd* (3.5, 3.5, 12.5)
H-5*ax*	1.90 1H, *dd* (5.0, 12.0)	1.71 1H, *dd* (3.0, 3.0, 12.0)	1.88 1H, *dd* (3.0, 12.0)	H-5*ax*	1.92 1H, *dd* (3.0, 13.0)	1.77 1H, *dd* (2.0, 12.0)
H-6 *ax*	1.07 1H, *ddd* (12.0, 13.0, 13.0)	1.24 1H, *ddd* (12.0, 13.0, 13.0)	1.42 1H, *ddd* (12.0, 13.0, 13.0)	H-6*ax*	1.41 1H, *ddd* (12.0, 13.0, 13.0)	1.33 1H, *ddd* (12.0, 12.0, 12.0)
eq	2.36 1H, *ddd* (3.0, 5.0, 13.0)	2.15 1H, *ddd* (3.0, 3.0, 13.0)	2.03 1H, *ddd* (3.0, 3.0, 13.0)	*eq*	2.05 1H, *ddd* (3.0, 3.0, 13.0)	2.96 1H, *br d* (12.0)
H-7*ax*	1.64 1H, *dddd* (3.0, 3.0, 13.0, 13.0)	1.57 1H, *dddd* (3.0, 3.0, 13.0, 13.0)	1.75 1H, *dddd* (3.0, 3.0, 13.0, 13.0)	H-7*ax*	1.67 1H, *dddd* (3.0, 3.0, 12.0, 12.0)	1.95 1H, *m*
H-8*ax*	1.21 1H, *dddd* (3.0, 13.0, 13.0, 13.0)	1.25 1H, *dddd* (3.0, 13.0, 13.0, 13.0)	1.64 1H, *dddd* (3.0, 13.0, 13.0, 13.0)	H-8*ax*	1.42 1H, *dddd* (3.0, 12.0, 12.0, 13.0)	1.33 1H, *br ddd* (12.0, 12.0, 12.0)
eq	2.05 1H, *dddd* (3.0, 3.0, 3.0, 13.0)	2.03 1H, *dddd* (3.0, 3.0, 3.0, 13.0)	2.06 1H, *br d* (13.0)	*eq*	1.98 1H, *dddd* (3.0, 3.0, 3.0, 13.0)	1.70 1H, *br d* (12.0)
H-9*ax*	1.66 1H, *ddd* (3.0, 13.0, 13.0)	1.65 1H, *ddd* (3.0, 13.0, 13.0)	1.28 1H, *ddd* (3.0, 13.0, 13.0)	H-9*ax*	1.34 1H, *ddd* (3.0, 13.0, 13.0)	1.36 1H, *br dd* (12.0, 12.0)
eq	3.06 1H, *ddd* (3.0, 3.0, 13.0)	2.29 1H, *ddd* (3.0, 3.0, 13.0)	3.11 1H, *ddd* (3.0, 3.0, 13.0)	*eq*	2.43 1H, *ddd* (3.0, 3.0, 13.0)	2.36 1H, *ddd* (3.0, 3.0, 12.0)
H_3-12	1.29 3H, *s*	1.41 3H, *s*	1.42 3H, *s*	H_3-12	1.45 3H, *s*	1.31 3H, *s*
H_3-13	1.38 3H, *s*	1.44 3H, *s*	1.44 3H, *s*	H_3-13	1.46 3H, *s*	1.49 3H, *s*
H-14a	–	3.91 1H, *d* (8.0)	4.07 1H, *d* (11.5)	H_3-14	1.00 3H, *s*	1.19 3H, *s*
b	–	4.30 1H, *d* (8.0)	4.19 1H, *d* (11.5)	H_3-15	–	1.36 3H, *s*
H_3-15	1.25 3H, *s*	1.21 3H, *s*	–	H-15a	4.65 1H, *br s*	–
H-15a	–	–	4.55 1H, *br s*	b	4.87 1H, *br s*	–
b	–	–	4.76 1H, *br s*	Glc H-1	5.07 1H, *d* (8.0)	5.04 1H, *d* (8.0)
Glc H-1	5.02 1H, *d* (8.0)	5.06 1H, *d* (8.0)	5.06 1H, *d* (8.0)			

[a] δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses].

Table 2
^{13}C NMR chemical shifts of **1**–**5** and **1a** (in pyridine-d_5, 125 MHz)[a]

	1	**1a**	**2**	**3**	**4**	**5**
C-1	74.25	74.22	76.65	81.04	84.50	79.42
C-2	30.32	30.32	30.47	33.12	71.83	29.89
C-3	35.40	35.43	39.10	34.79	44.28	42.02
C-4	83.25	83.16	82.90	149.30	148.80	71.41
C-5	49.79	49.88	50.16	47.19	48.61	53.37
C-6	26.18	26.19	25.28	25.07	24.96	21.83
C-7	45.57 (−1.7)	47.29	46.85	48.32	48.46	48.21
C-8	25.09	24.99	23.58	22.33	22.23	23.87
C-9	28.35	28.32	28.96	32.57	38.01	41.99
C-10	54.71	54.71	49.03	42.29	39.98	39.84
C-11	79.46 (+8.3)	71.17	79.69	79.50	79.55	80.27
C-12	23.38 (−3.5)	26.91	23.85	23.96	24.02	23.83
C-13	24.73 (−3.2)	27.92	24.69	24.81	24.78	25.50
C-14	177.57	177.45	68.41	60.30	12.00	14.08
C-15	20.55	20.49	21.61	106.73	107.94	23.34
Glc-1	98.55		98.67	98.72	98.73	98.54
Glc-2	75.29		75.39	75.38	75.43	75.47
Glc-3	78.83		78.90	78.95	79.95	78.74
Glc-4	71.86		71.93	71.98	72.01	72.14
Glc-5	78.16		78.05	78.03	78.05	78.58
Glc-6	63.11		63.14	63.12	63.15	63.06

[a] δ in ppm from TMS: Δδ (δ glucoside − δ aglycone) are given in parentheses.

monoglucoside of sesquiterpenoid. From the results of heteronuclear multiple bond connectivity (HMBC) spectral data (see Section 3), the aglycone of **1** was considered to be 1,11-dihydroxy-eudesmane with a lactone ring between C-4 and C-14. The position of the glucosyl unit was found to be C-11 by the observed cross-peak between Glc H-1/C-11 in the HMBC spectrum. The stereochemistry of **1** was examined by the results of its nuclear Overhauser and exchange spectroscopy (NOESY) spectrum. The observed NOE interactions between H-1/H-3*ax*, H-5, H-9*ax*, and between H-5/H-3*ax*, H-7 suggested that the configuration of H-1, H-5 and H-7 was α-axial, and the NOE interactions between H_3-15/H-3*ax*, H-5*ax* suggested that the A-B ring juncture was *trans*, and the configuration of C-4 methyl was β-equatorial (Fig. 1). Consequently, **1** was found to be 1β,11-dihydroxyeudesman-14,4-olide 11-*O*-β-D-glucopyranoside. Furthermore, the absolute configuration at the C-1 position in **1** was determined by means of the modified Mosher's method (Ohtani et al., 1991; Kitajima et al., 2002). Treatment of **1a** with *S*-(−)- and *R*-(+)-2-methoxy-2-trifluoromethyl phenyl acetate acid (MTPA) chloride gave the 1-*O*-esters of *S*-(-)-MTPA (**1a-*S***) and *R*-(+)-MTPA (**1a-*R***), respectively. Signals

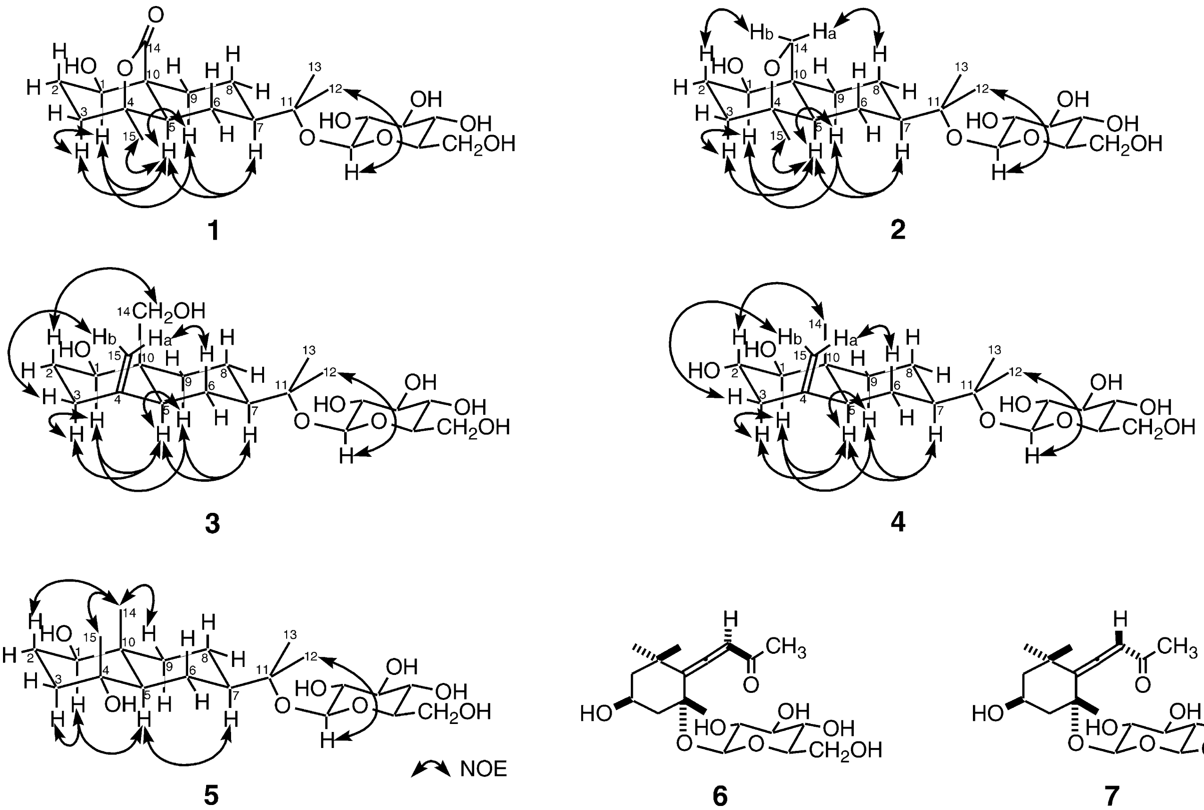
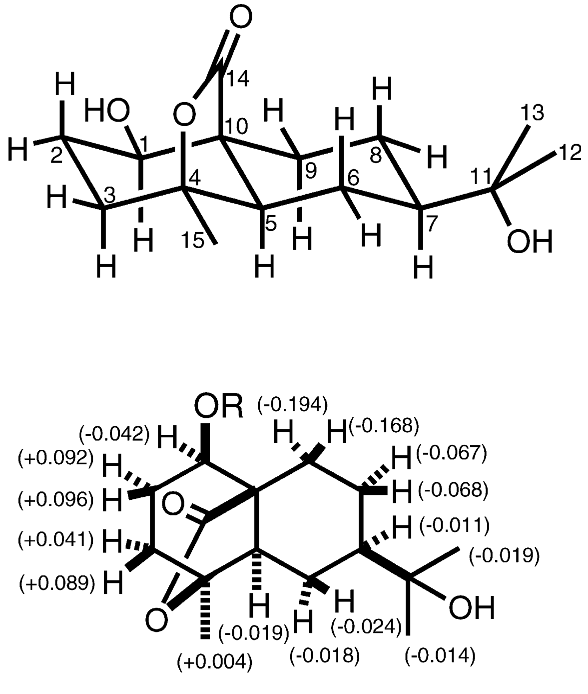


Fig. 1. Structures of **1**–**7**, and NOE interactions observed in the NOESY spectra of **1**–**5**.

due to protons on C-2, C-3 and C-15 of **1a-*S*** appeared at lower field than those of **1a-*R***, while the proton signals due to the C-5 to C-9, C-12 and C-13 of **1a-*S*** were observed at higher field than those of **1a-*R*** (Fig. 2). So, the absolute configuration at C-1 of **1** is assigned as *R*. Therefore, the absolute structure of **1** was described as shown in Fig. 1 [(1*R*,4*S*,5*R*,7*R*,10*S*)-1,11-dihydroxyeudesman-14,4-olide 11-*O*-β-D-glucopyranoside].

Celerioside B (**2**; $C_{21}H_{36}O_8$, an amorphous powder, $[\alpha]_D^{23}$ −9°) showed $[M+Na]^+$ and $[M+H]^+$ ion peaks at *m*/*z* 439 and 417, and an $[M\text{-}C_6H_{12}O_6+H]^+$ ion peak at *m*/*z* 237 in the positive-ion FAB-MS. It showed similar NMR spectral features with those of **1** (Tables 1 and 2) except for the presence of signals of an oxygenated methylene, instead of a carbonyl carbon. From the analysis of the HMBC spectral data (see Section 3), **2** was suggested to be a deoxo-derivative of **1**. Furthermore, the observed NOE interactions in its NOESY spectrum (Fig. 1) suggested that the configuration of H-1 and H-5 and H-7 was α-axial, C-4 methyl was α-equatorial, and the A-B ring juncture was *trans*, the same as **1**. Therefore, **2** was shown in Fig. 1 [1β,11-dihydroxyeudesman-4,14-oxide 11-*O*-β-D-glucopyranoside].

Celeroside C (**3**; $C_{21}H_{36}O_8$, an amorphous powder, $[\alpha]_D^{23}$ +20°) showed $[M+Na]^+$, $[M+H]^+$ and $[M\text{-}C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 439, 417 and 237 in the positive FAB-MS. The NMR spectral data (Tables 1 and 2) of **3** showed the presence of two *tert*-methyls, one hydroxymethyl, five methylenes, three methines (one oxygenated), two quaternary carbons (one oxygenated) and one terminal-methylene group, in addition to a β-glucopyranosyl group (Tables 1 and 2). From comparison of NMR spectral data with those of **2**, and analysis of its HMBC spectral data (see Section 3), **3** was the β-glucopyranoside of eudesm-4(15)-ene-1,11,14-triol. The position of the β-glucosyl unit was found to be C-11 from the H-C long-range correlation between Glc H-1/C-11 in the HMBC spectrum. The observed NOE interactions between H-1/H-3*ax*, H-5, H-9*ax*, and between H-5/H-7, H-9*ax* in its NOESY spectrum suggested that the configuration of H-1, H-5 and H-7 was α-axial, and the NOE interactions between H_2-14/H-2*ax* suggested that the A-B ring juncture was *trans*. So, **3** was described as shown in Fig. 1 [eudesman-4(15)-ene-1β,11,14-triol 11-*O*-β-D-glucopyranoside].



1a: R=H, **1a-*S***: R=*S*-(-)-MTPA, **1a-*R***: R=*R*-(+)-MTPA

Fig. 2. Δδ Values in Hz [=δ*S*-(−)−δ*R*-(+)] of **1a-MTPA** ester.

Celerioside D (**4**; $C_{21}H_{36}O_8$, an amorphous powder, $[\alpha]_D^{23}$ +13°) showed an $[M+H]^+$ ion peak at *m*/*z* 417, and an $[M\text{-}C_6H_{12}O_6+H]^+$ ion peak at *m*/*z* 237 in the positive FAB-MS. By comparison of its NMR spectral data with those of **3** (Tables 1 and 2), **4** was considered to be a dihydroxylated form of eudesm-4(15)-ene-11-ol β-D-glucopyranoside. The location of the two hydroxyl groups of **4** was found to be C-1 and C-2, respectively, from the analysis of the HMBC spectrum (see Section 3), and the broad H-1 and H-2 (δ 3.54; *d*, *J*=9.5 Hz, and δ 4.08; *ddd*, *J*=5.0, 9.5, 13.0 Hz) signals suggested the configuration of two hydroxyl groups were equatorial. The NOE interactions between H-1/H-3*ax*, H-5, H-9*ax*, between H-5/H-3*ax*, H-7, H-9*ax* in its NOESY spectrum suggested that the configurations of H-1, H-5 and H-7 were α-axial, and the NOE interaction between H-2*ax*/H_3-14 indicated that the configuration of H-2 and C-10 methyl was β-axial. Then, **4** was described as shown in Fig. 1 [eudesman-4(15)-ene-1β,2α,11-triol 11-*O*-β-D-glucopyranoside].

Celeroside E (**5**; $C_{21}H_{38}O_8$, an amorphous powder, $[\alpha]_D^{23}$ +9°) showed $[M+K]^+$, $[M+Na]^+$ and $[M\text{-}C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 457, 441 and 239 in the positive FAB-MS. The NMR spectral data (Tables 1 and 2) of **5** showed the presence of four *tert*-methyls, five methylenes, three methines (one oxygenated) and three quaternary carbons (two oxygenated), in addition to a β-glucopyranosyl group (Tables 1 and 2). From comparison of NMR spectral data with those of **3** and **4**, and analysis of its HMBC spectral data (see Section 3), **5** was eudesm-4(15)-ene-1,4,14-triol 11-*O*-β-D-glucopyranoside. As the NOE interactions between H-1/H-3*ax*, H-5, between H-5/H-7 in its NOESY spectrum suggested that the configurations of H-1, H-5 and H-7 were α-axial, and the NOE interaction between H_3-14/H-2*ax*, H_3-15 suggested that the configuration of C-4 and C-10 methyl was β-axial. Therefore, **5** was as shown in Fig. 1 [eudesmane-1β,4α,11-triol 11-*O*-β-D-glucopyranoside].

Norcarotenoid glucosides **6** and **7** were identified as citroside A and B (Umehara et al., 1998), respectively.

Table 3
^{1}H NMR chemical shifts of **8**–**10** (in pyridine-d_5, 500 MHz)[a]

	8	**9**		**10**
H-3	5.60 1H, *dd* (4.0, 7.5)	5.57 1H, *dd* (4.0, 6.5)	H-3	3.92 1H, *ddd* (4.5, 8.5, 8.5)
H-4	7.56 1H, *dd* (1.5, 7.5)	7.52 1H, *dd* (1.5, 7.5)	H-4*ax*	0.90 1H, *dddd* (3.0, 13.0, 13.0, 13.0)
H-5	7.59 1H, *ddd* (1.5, 7.5, 7.5)	7.56 1H, *ddd* (1.5, 7.5, 7.5)	*eq*	1.79 1H, *m*
H-6	7.46 1H, *ddd* (1.5, 7.5, 7.5)	7.44 1H, *ddd* (1.5, 7.5, 7.5)	H-5*ax*	1.23 1H, *m*
H-7	7.96 1H, *dd* (1.5, 7.5)	7.95 1H, *dd* (1.5, 7.5)	*eq*	1.60 1H, *m*
H_2-1′	1.90 1H, *m*	1.83 1H, *m*	H-6*ax*	1.91 1H, *m*
	2.52 1H, *m*	2.52 1H, *m*	*eq*	2.05 1H, *dddd* (3.0, 3.0, 5.0, 16.5)
H_2-2′	1.71 1H, *m*	1.68 1H, *m*	H-7	6.71 1H, *dd* (3.0, 6.5)
	1.82 1H, *m*	1.83 1H, *m*	H-9	2.33 1H, *ddd* (4.0, 8.5, 13.0)
H-3′	4.02 1H, *ddd* (6.0, 6.0, 12.0)	3.99 1H, *ddd* (6.0, 6.0, 12.0)	H_2-1′	1.91 1H, *m*
H_3-4′	1.32 3H, *d* (6.0)	1.37 3H, *d* (6.0)		2.17 1H, *dddd* (4.5, 13.0, 13.0, 13.0)
Glc H-1	4.85 1H, *d* (8.0)	4.81 1H, *d* (7.5)	H_2-2′	1.79 2H, *m*
Api H-1		5.79 1H, *d* (2.5)	H-3′	4.05 1H, *ddd* (6.0, 6.0, 12.0)
			Glc H-1	4.93 1H, *d* (7.5)

[a] δ in ppm from TMS coupling constants (J) in Hz are given in parentheses.

Table 4
H NMR chemical shifts of **8**–**10** and **8a** (in pyridine-d_5, 125 MHz)[a]

	8	**8a**	**9**	**10**
C-1	170.61	170.68	170.58	169.91
C-3	82.05	82.06	82.07	85.55
C-4	122.73	122.59	122.70	25.29
C-5	134.27	134.30	134.23	20.93
C-6	129.33	129.35	129.29	25.06
C-7	125.51	125.56	125.50	134.79
C-8	126.55	126.60	126.57	131.89
C-9	150.66	150.77	150.71	43.14
C-1′	30.79	31.86	31.09	30.87
C-2′	32.37 (−2.3)	34.64	32.71	33.37
C-3′	75.99 (+9.2)	66.74	76.38	76.32
C-4′	22.19 (−2.1)	24.26	22.41	22.38
Glc-1	104.32		104.48	104.64
Glc-2	75.38		75.32	75.49
Glc-3	78.61		78.63	78.66
Glc-4	71.71		71.92	71.79
Glc-5	78.32		77.02	78.39
Glc-6	62.85		69.01	62.93
Api-1			111.14	
Api-2			77.85	
Api-3			80.48	
Api-4			75.05	
Api-5			65.63	
Api-6			69.01	

[a] δ in ppm from TMS: $\Delta\delta$ (δ glucoside − δ aglycone) are given in parentheses.

Celephthalide A (**8**; $C_{18}H_{24}O_8$, an amorphous powder, $[\alpha]_D^{23}$ −43°) showed $[M+Na]^+$, $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m*/*z* 391, 369 and 207 in the positive-ion FAB-MS, and $[M-H]^-$ and $[M-C_6H_{12}O_6-H]^-$ ion peaks at *m*/*z* 367 and 187 in the negative FAB-MS. Enzymatic hydrolysis of **8** with β-glucosidase gave an aglycone (**8a**; $C_{12}H_{14}O_3$, an amorphous powder, $[\alpha]_D^{23}$ −41°) and D-glucose. The NMR spectral data (Tables 3 and 4) of **8** was built up with one β-glucopyranosyl group, one 1,2-substituted benzene ring, one *sec*-methyl, two methylenes, two oxygenated methines and a lactone group, and suggested it to be a monoglucoside of phthalide. From the results of HMBC spectral data (see Section 3), the planar structure of the aglycone moiety was concluded to be 3′-hydroxy-3-butyl phthalide, and the position of the glucosyl unit was found to be C-3′. The stereochemistry of C-3′ of the aglycone moiety of **8** was examined by the empirical rule of ^{13}C-NMR glycosylation shift (Kasai et al., 1977; Tori et al., 1977). By comparison of the ^{13}C glycosylation shift values [$\Delta\delta$ (δ glucoside–δ aglycone)] of the α- and β-carbon and the chemical shift of Glc C-1 with those of 2*R*- and 2*S*-form of 2-pentanol β-D-glucopyranosides (Kijima et al., 1996), the absolute configuration at C-3′ of **8** was indicated to be *S* [C-3′ (α-carbon), $\Delta\delta+9.2$; *R*-form, $\Delta\delta+6.8$; *S*-form, $\Delta\delta+9.9$: C-2′ (β-carbon), $\Delta\delta$ −2.3; β-pro-*R*-side carbon of *R*-form, $\Delta\delta$ −1.8; β-pro-*S*-side carbon of *S*-form, $\Delta\delta$−2.5: C-4′ (β-carbon), $\Delta\delta$ −2.1; β-pro-*S*-side carbon of *R*-form, $\Delta\delta$ −3.8; β-pro-*R*-side carbon of *S*-form, $\Delta\delta$−1.5: glucosyl C-1, δ 104.32; *R*-form, δ 102.1; *S*-form, δ 104.0: (Table 4)]. From these facts, **8** was (3′*S*)-3′-hydroxy-3-butyl phthalide β-D-glucopyranoside as described in Fig. 3.

Celephthalide B (**9**; $C_{23}H_{32}O_{12}$, an amorphous powder, $[\alpha]_D^{23}$ −76°) showed $[M+K]^+$, $[M+Na]^+$, $[M+H]^+$ and $[M-C_{11}H_{18}O_9+H]^+$ ion peaks at *m*/*z* 539, 523, 501 and 207 in the positive FAB-MS, and an $[M-H]^-$ ion peak at *m*/*z* 499 in the negative FAB-MS. It showed similar NMR spectral features with those of **8** except the signals due to a β-D-apiofuranosyl group (Tables 3 and 4), and suggested it to be a β-D-apiofuranoside of **8**. The additional apiofuranosyl unit was located at C-6 of the glucose from the downfield shift of the glucosyl C-6 carbon (6.2 ppm) and the observed cross-peak between Api H-1/Glc C-6 in the HMBC spectrum. Comparison of the $[M]_D$ value of **9** with that of **8** ($[M]_D$ value of

9−$[M]_D$ value of **8** = 224°) suggested that the apiose was D-form (Hulyalkar et al., 1965). Therefore, **9** was (3′*S*)-3′-hydroxy-3-butyl phthalide β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside as described in Fig. 3.

Celephthalide C (**10**; $C_{18}H_{28}O_8$, an amorphous powder, $[\alpha]_D^{23}$ −56°) showed $[M+Na]^+$, $[M+H]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 395, 373 and 193 in the positive FAB-MS, and an $[M-H]^-$ ion peak at *m*/*z* 371 in the negative FAB-MS. The NMR spectral data (Tables 3 and 4) of **10** showed the presence of one trisubstituted double bond, one *sec*-methyl, five methylenes, three methines (two of them were oxygenated) and a lactone group, in addition to a β-glucopyranosyl group (Tables 3 and 4). From comparison of NMR spectral data with those of **8**, and analysis of its ^{1}H-^{1}H correlation spectroscopy (COSY) spectral data, **10** was indicated to be β-glucopyranoside of 3′-hydroxysedanolide. The position of the β-glucosyl unit was found to be C-3′ from the observed NOE interactions between Glc H-1/H-3′ in its NOESY spectrum, and the configuration of both H-3 and H-9 was considered to be axial, by the NOE interactions between H-3/H-4*ax* and H-5*ax*/H-9 (Fig. 3). The configuration at C-3′ of **10** was concluded to be *S* by comparison of its C-2′, C-3′, C-4′ chemical shifts with those of **8**. So, **10** was elucidated as (3*S*)-3′-hydroxysedanolide β-D-glucopyranoside as shown in Fig. 3.

Aromatic compound glycosides **11**–**16**, and a lignan glucoside **17** were identified as leonuriside A (Otsuka et al., 1989), 4-hydroxy-3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (Kitajima et al., 1998), icariside F$_2$, icariside D$_1$ (Miyase et al., 1987), syringin, junipediol A 4-*O*-β-D-glucopyranoside (Matsumura et al., 2002) and (7*S*,8*R*,8′*R*)-(+)-lariciresinol 9-*O*-β-D-glucopyranoside (Satake et al., 1978), respectively.

3. Experimental

3.1. General

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. FAB-MS were recorded with a JEOL HX-110 spectrometer using glycerol as matrix. ^{1}H and ^{13}C NMR spectra were taken on JEOL A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. Column chromatography (C. C.) was carried out under TLC monitoring using Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 µm, Pharmacia), Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck

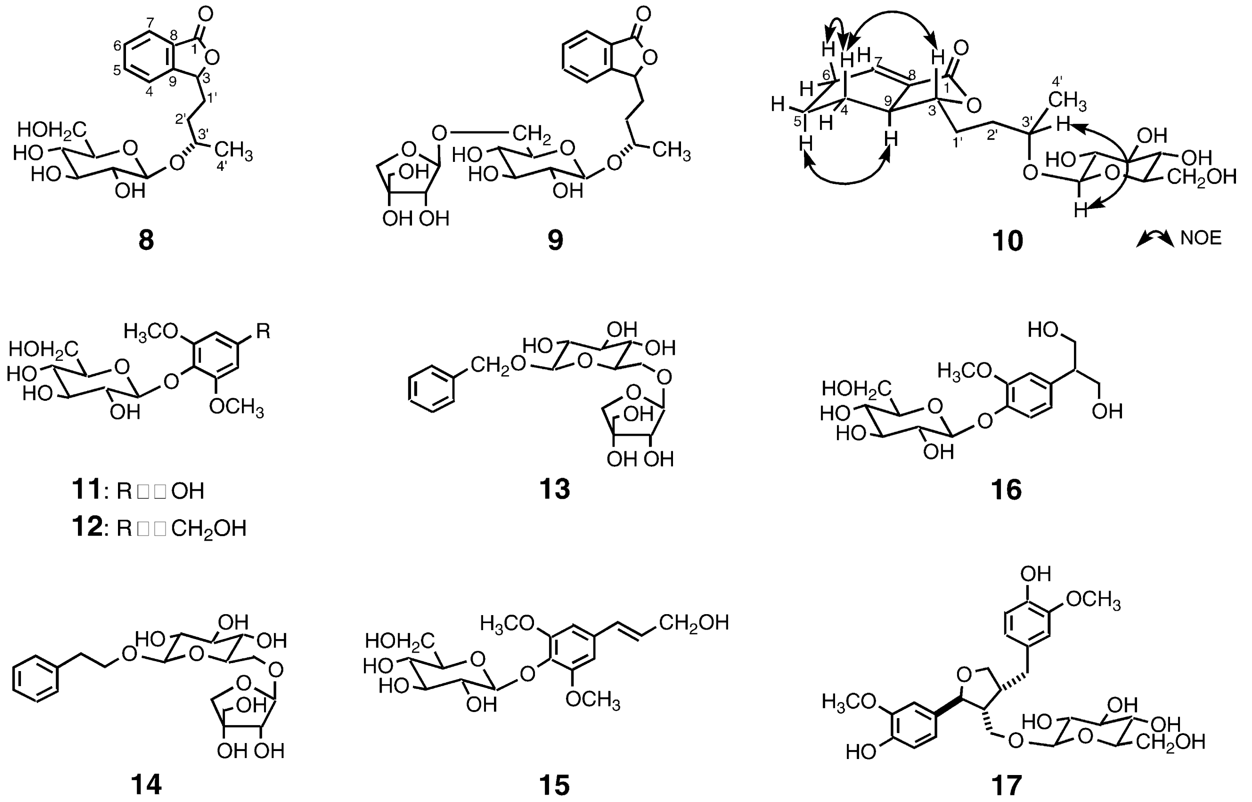


Fig. 3. Structures of **8**–**17**, and NOE interactions observed in the NOESY spectra of **10**.

5721) and spots were detected with *p*-anisaldehyde–H_2SO_4 reagent. HPLC separation was carried out with Symmetryprep C_{18} 7μm (Waters; column size, 7.8 × 300 mm; ODS), carbohydrate analysis (Waters; column size, 3.9 × 300 mm; CHA) columns.

3.2. Extraction and separation

Commercial celery seed (the fruit of *Apium graveolens* L.; purchased from Asaoka Spices Ltd., Lot. No. 00011801; 2.0 kg) was extracted with methanol–H_2O (7:3) (6 l × 4), and the extract (282.6 g) was partitioned into ether–water and ethyl acetate–water, respectively. The aqueous portion (182.1 g) was purified further over Amberlite XAD-II ($H_2O \rightarrow MeOH$) to give water (114.1 g) and methanol eluate (68.0 g) fractions.

The methanol fraction was subjected to Sephadex LH-20 chromatography (MeOH) to give five fractions (frs. A–E). Fraction B (26.39 g) was applied to silica gel cc [$CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→7:3:0.5)→ MeOH] to give 15 fractions (frs. B_1–B_{15}). Fraction B_3 (0.97 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 17 fractions (frs. $B_{3\text{-}1}$–$B_{3\text{-}17}$). Fraction $B_{3\text{-}8}$ was subjected to HPLC [ODS, MeCN–H_2O (3:17)] to give **8** (180 mg), and fr. $B_{3\text{-}14}$ was subjected to HPLC [ODS, MeOH–H_2O (2:3)] to give **10** (19 mg). Fraction B_4 (0.72 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give ten fractions (frs. $B_{4\text{-}1}$–$B_{4\text{-}10}$), and fr. $B_{4\text{-}10}$ was subjected to HPLC [ODS, MeOH–H_2O (2:3) and CHA, MeCN–H_2O (24:1)] to give **17** (12 mg). Fraction B_6 (1.13 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 17 fractions (frs. $B_{6\text{-}1}$–$B_{6\text{-}17}$), and fr. $B_{6\text{-}5}$ was subjected to HPLC [ODS, MeCN–H_2O (1:19)] to give **11** (16 mg) and **12** (31 mg). Fraction $B_{6\text{-}7}$ (0.92 g) was subjected to HPLC [ODS, MeCN–H_2O (1:9)] to give **15** (15 mg), and fr. $B_{6\text{-}6}$ was subjected to HPLC [ODS, MeCN–H_2O (1:4), and CHA, MeCN–H_2O (24:1)] to give **3** (30 mg). Fraction $B_{6\text{-}8}$ was subjected to HPLC [ODS, MeOH–H_2O (2:3) and CHA, MeCN–H_2O (24:1)] to give **16** (10 mg) and **7** (5 mg), $B_{6\text{-}10}$ was subjected to HPLC [ODS, MeCN–H_2O (1:9) and CHA, MeCN–H_2O (24:1)] to give **5** (14 mg), and fr. $B_{6\text{-}15}$ was subjected to HPLC [ODS, MeOH–H_2O (2:3)] to give **9** (9 mg) and **4** (5 mg). Fraction B_7 (1.44 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 15 fractions (frs. $B_{7\text{-}1}$–$B_{7\text{-}15}$), and fr.$B_{7\text{-}7}$ was subjected to HPLC [ODS, MeCN–H_2O (1:9)] to give **13** (15 mg) and **6** (60 mg). Fraction $B_{7\text{-}11}$ was subjected to HPLC [ODS, MeOH–H_2O (2:3)] to give **2** (15 mg) and **1** (40 mg). Fraction B_8 (1.33 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 13 fractions (frs. $B_{8\text{-}1}$–$B_{8\text{-}13}$), and fr.$B_{8\text{-}3}$ was subjected to HPLC [ODS, MeOH–H_2O (1:19)] to give **14** (10 mg).

The following compounds were identified by comparison with authentic compounds or published physical and spectral data: citroside A (**6**), citroside B (**7**), leonuriside A (**11**), 4-hydroxy-3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (**12**), icariside F_2 (**13**), icariside D_1 (**14**), syringin (**15**), junipediol A 4-*O*-β-D-glucopyranoside (**16**) and (7*S*,8*R*,8′*R*)-(+)-lariciresinol 9-*O*-β-D-glucopyranoside (**17**).

*3.3. Celerioside A (**1**)*

Colorless needles (MeOH), mp 233–225 °C, $[\alpha]_D^{23}$ +19° (c=2.3, MeOH). Positive FAB-MS *m/z*: 453.2099 $[M+Na]^+$ (Calcd for $C_{21}H_{34}NaO_9$; 453.2100), 431 $[M+H]^+$, 251 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). Negative FAB-MS *m/z*: 429 $[M\text{-}H]^+$ (base). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2. HMBC Correlations: H-1*ax*/C-2, C-5, C-9, C-10, C-14; H-2*ax*/ C-1, C-3, C-10; H-2*eq*/C-1, C-3, C-4, C-10; H-3*ax*/C-1, C-2, C-5; H-3*eq*/C-1, C-2, C-4, C-5, C-15; H-5*ax*/C-1, C-3, C-6, C-7, C-10, C-14; H-6*ax*/C-4, C-5, C-7, C-8, C-10, C-11; H-6*eq*/C-5, C-7, C-8, C-10, C-11; H-7*ax*/C-5, C-11, C-12, C-13; H-8*ax*/C-6, C-7, C-9, C-10, C-11; H-8*eq*/C-6, C-10; H-9*ax*/C-7, C-8, C-10, C-14; H-9*eq*/C-5, C-7, C-8, C-10, C-14; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H_3-15/C-3, C-4, C-5; Glc H-1/C-11.

*3.4. Enzymatic hydrolysis of **1***

A mixture of **1** (8 mg) and β-glucosidase (3 mg, Toyobo Co. Ltd., Lot 52275) in water (5 ml) was shaken in a water bath at 37 °C for 30 days. The mixture was concentrated in vacuo to dryness and the residue was purified further silica gel [$CHCl_3$–MeOH (12:1) and $CHCl_3$–MeOH–H_2O (1:1:0.1)] to afford **1a** (5 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (Waters), detector; JASCO RI-930 detector and JASCO OR-990 chiral detector, solv.; MeCN–H_2O (17:3), 2 ml/min; t_R 4.50 min (same location as that of D-glucose)] show the presence of D-glucose.

*3.5. (1R,4S,5R,7R,10S)-1,11-Dihydroxyeudesman-14,4-olide (**1a**)*

An amorphous powder, $[\alpha]_D^{23}$ +30° (c=0.3, MeOH). ^1H NMR (pyridine-d_5, 500 MHz) δ: 3.87 (1H, *dd*, J=6.0, 10.5 Hz, H-1*ax*), 3.10 (1H, *ddd*, J=3.0, 3.0, 12.5 Hz, H-9*eq*), 2.24 (1H, *brddd*, J=6.0, 6.5, 13.0 Hz, H-2*eq*), 2.19 (1H, *ddd*, J=3.0, 3.0, 12.5 Hz, H-6*eq*), 2.06 (1H, *dddd*, J=3.0, 3.0, 3.0, 12.5 Hz, H-8*eq*), 2.00 (1H, *dddd*, J=6.5, 10.5, 13.0, 13.0 Hz, H-2*ax*), 1.83 (1H, *brdd*, J=6.5, 13.0 Hz, H-3*eq*), 1.69 (1H, *dd*, J=5.5, 12.5 Hz, H-5*ax*), 1.62 (1H, *ddd*, J=3.0, 12.5, 12.5 Hz, H-9*ax*), 1.62 (1H, *ddd*, J=6.5, 13.0, 13.0 Hz, H-3*ax*), 1.43 (1H, *dddd*, J=3.0, 3.0, 12.5, 12.5 Hz, H-7*ax*), 1.31 (1H, *dddd*, J=3.0, 12.5, 12.5, 12.5 Hz, H-8*ax*), 1.32 (3H, *s*, H_3-13), 1.29 (3H, *s*, H_3-12), 1.26 (3H, *s*, H_3-15), 1.18

(1H, *ddd*, J = 12.5, 12.5, 12.5 Hz, H-6*ax*). For ^{13}C-NMR spectrum, see Table 2.

3.6. 1-O-*MTPA ester of* **1a**

A solution of **1a** (2 mg) in a mixture of $CHCl_3$ and pyridine (each 0.5 ml) was treated with *R*-(−)-MTPA-chloride (5 drops) and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with EtOAc, and the EtOAc extract was subjected to silica gel cc (hexane–EtOAc = 4:1) to give 1-*O*-*S*-(−)-MTPA ester of **1a-*S*** (1a-*S*-(−)-MTPA) In a similar procedure, **1a-*R*** (1a-*R*-(+)-MTPA) was obtained with *S*-(+)-MTPA-chloride.

3.7. *Celerioside B (**2**)*

An amorphous powder, $[\alpha]_D^{23}$ −9° (c = 0.8, MeOH). Positive FAB-MS *m*/*z*: 439 $[M+Na]^+$, 417.2488 $[M+H]^+$ (Calcd for $C_{21}H_{37}O_8$; 417.2488), 399 $[M\text{-}H_2O+H]^+$, 237 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). Negative FAB-MS *m*/*z*: 415 $[M\text{-}H]^+$ (base). For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. HMBC Correlations: H-1*ax*/C-2, C-5, C-9, C-10, C-14; H-2*ax*/ C-1, C-3, C-4, C-10; H-2*eq*/C-1, C-3, C-4, C-10; H-3*ax*/C-1, C-4, C-15; H-3*eq*/C-1, C-2, C-4, C-5; H-5*ax*/C-1, C-4, C-6, C-9, C-15; H-6*ax*/C-4, C-5, C-7, C-8, C-11; H-6*eq*/C-4, C-5, C-7, C-8, C-10; H-7*ax*/C-5, C-8, C-12, C-13; H-8*ax*/C-6, C-7, C-9, C-11; H-8*eq*/C-6, C-10; H-9*ax*/C-1, C-5, C-7, C-8, C-10, C-14; H-9*eq*/C-5, C-7, C-8, C-10, C-14; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H-14a/C-1, C-4, C-5, C-9, C-10; H-14b/C-1, C-4, C-5, C-10; H_3-15/C-3, C-4, C-5; Glc H-1/C-11.

3.8. *Celerioside C (**3**)*

An amorphous powder, $[\alpha]_D^{23}$ +20° (c = 2.4, MeOH). Positive FAB-MS *m*/*z*: 439.2299 $[M+Na]^+$ (Calcd for $C_{21}H_{36}NaO_8$; 439.2308), 417 $[M+H]^+$, 399 $[M\text{-}H_2O+H]^+$, 237 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. HMBC correlations: H-1*ax*/C-10; H-2*ax*/ C-1, C-3, C-4, C-10; H-2*eq*/C-1, C-3, C-4, C-10; H-3*ax*/C-1, C-2, C-4, C-15; H-3*eq*/C-1, C-2, C-4, C-5; H-5*ax*/C-1, C-3, C-4, C-6, C-7, C-10, C-14, C-15; H-6*ax*/C-4, C-5, C-7, C-10, C-11; H-6*eq*/C-4, C-5, C-7, C-8, C-10; H-7*ax*/C-5, C-8, C-9, C-11, C-12, C-13; H-8*ax*/C-6, C-7, C-9, C-10, C-11; H-8*eq*/C-6, C-7, C-9, C-10; H-9*ax*/C-1, C-5, C-7, C-8, C-10, C-14; H-9*eq*/C-5, C-7, C-8, C-10, C-14; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H-14a/C-1, C-10; H-14b/C-1, C-9, C-10; H_2-15/C-3, C-4, C-5; Glc H-1/C-11.

3.9. *Celerioside D (**4**)*

An amorphous powder, $[\alpha]_D^{23}$ +13° (c = 0.3, MeOH). Positive FAB-MS *m*/*z*: 417.2477 $[M+H]^+$ (Calcd for $C_{21}H_{37}O_8$; 417.2489), 399 $[M\text{-}H_2O+H]^+$, 237 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). For ^{1}H NMR and ^{13}C NMR spectra, see Tables 1 and 2. HMBC correlations: H-1*ax*/C-2, C-3, C-5, C-9, C-10, C-14; H-2*ax*/ C-1; H-3*ax*/C-1, C-2, C-4, C-15; H-3*eq*/C-1, C-2, C-4, C-5, C-15; H-5*ax*/C-4, C-6, C-10, C-14, C-15; H-6*ax*/C-5, C-7, C-8, C-10, C-11; H-6*eq*/C-5, C-7, C-8, C-10; H-7*ax*/C-5, C-11, C-12, C-13; H-8*ax*/C-9; H-8*eq*/C-6, C-10; H-9*ax*/C-1, C-5, C-8, C-10, C-14; H-9*eq*/C-5, C-7, C-8, C-10; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H_3-14/C-1, C-5, C-9, C-10; H-15a/C-3, C-4, C-5; H-15b/C-3, C-4, C-5; Glc H-1/C-11.

3.10. *Celerioside E (**5**)*

An amorphous powder, $[\alpha]_D^{23}$ +9° (c = 2.4, MeOH). Positive FAB-MS *m*/*z*: 457.2204 $[M+K]^+$ (Calcd for $C_{21}H_{38}KO_8$; 457.2203), 441.2478 $[M+Na]^+$ (Calcd for $C_{21}H_{38}NaO_8$; 441.2464), 239 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. H-1*ax*/C-3, C-9, C-10, C-14; H-3*ax*/C-1, C-2, C-4, C-15; H-3*eq*/C-1, C-2, C-4, C-5, C-10, C-15; H-5*ax*/C-1, C-3, C-4, C-6, C-7, C-10, C-14, C-15; H-6*ax*/C-11; H-6*eq*/C-4, C-5, C-7, C-8, C-10; H-8*eq*/C-6, C-7, C-10; H-9*ax*/C-14; H-9*eq*/C-5, C-7, C-8, C-10, C-14; H_3-12/C-7, C-11, C-13; H_3-13/C-7, C-11, C-12; H_3-14/C-1, C-5, C-9, C-10; H_3-15/C-3, C-4, C-5; Glc H-1/C-11.

3.11. *Celephtalide A (**8**)*

An amorphous powder, $[\alpha]_D^{23}$ −43° (c = 3.0, MeOH). Positive FAB-MS *m*/*z*: 391.1369 $[M+Na]^+$ (Calcd for $C_{18}H_{24}NaO_8$; 391.1369), 369 $[M+H]^+$, 207 $[M\text{-}C_6H_{10}O_5+H]^+$ (base). Negative FAB-MS *m*/*z*: 367 $[M\text{-}H]^-$, 187 $[M\text{-}C_6H_{12}O_6\text{-}H]^-$ (base). For ^{1}H NMR and ^{13}C NMR spectra, see Tables 3 and 4. HMBC correlations: H-3/C-1, C-4, C-8, C-9, C-1′, C-2′; H-4/C-3, C-6, C-8, C-9; H-5/C-6, C-7, C-9; H-6/C-4, C-5, C-8; H-7/C-1, C-5, C-9; H_2-1′/C-3, C-9, C-2′, C-3′; H_2-2′/C-3, C-1′, C-3′, C-4′; H-3′/C-1′, C-2′, C-4′, Glc C-1; H_3-4′/C-2′, C-3′; Glc H-1/C-3′.

3.12. *Enzymatic hydrolysis of **8***

A mixture of **8** (18 mg) and β-glucosidase (3 mg) in water (5 ml) was shaken in a water bath at 37 °C for 10 days. The mixture was chromatographed over silica gel [$CHCl_3$–MeOH (24:1) and $CHCl_3$–MeOH–H_2O (1:1:0.1)] to afford **8a** (7 mg) and a sugar fraction. From the sugar fraction, D-glucose was detected as described for **1**.

3.13. *(3S)-3′-Hydroxy-3-butyl phthalide (**8a**)*

An amorphous powder, $[\alpha]_D^{23}$ −41° (c = 0.6, MeOH). ^{1}H NMR (pyridine-d_5, 270 MHz) δ: 7.98 (1H, *d*, J = 7.5

Hz, H-7), 7.61 (1H, *ddd*, $J=1.5, 7.5, 7.5$ Hz, H-5), 7.48 (1H, *d*, $J=7.5$ Hz, H-4), 7.47 (1H, *ddd*, $J=1.5, 7.5, 7.5$ Hz, H-6), 5.64 (1H, *dd*, $J=4.0, 7.0$ Hz, H-3), 2.48 (1H, *m*, H-1′b), 1.68–1.95 (3H, m, H-1′a, H_2-2′), 1.28 (3H, *d*, $J=6.5$, H-4′). ^{13}C-NMR: Table 4.

3.14. Celephthalide B (**9**)

An amorphous powder, $[\alpha]_D^{23}$ −76° ($c=0.5$, MeOH). Positive FAB-MS *m/z*: 539.1544 [M+K]$^+$ (Calcd for $C_{23}H_{32}KO_{12}$; 539.1531), 523.1818 [M+Na]$^+$ (Calcd for $C_{23}H_{32}NaO_{12}$; 523.1792), 501.1994 [M+H]$^+$ (Calcd for $C_{23}H_{33}O_{12}$; 501.1972), 207 [M-$C_{11}H_{18}O_9$+H]$^+$ (base). Negative FAB-MS *m/z*: 499 [M-H]$^-$ (base). For ^1H NMR and ^{13}C NMR spectra, see Tables 3 and 4. HMBC correlations: H-3/C-1, C-8, C-9, C-1′, C-2′; H-4/C-3, C-6, C-8; H-5/C-6, C-7, C-9; H-6/C-4, C-8; H-7/C-1, C-5, C-9; H_2-1′/C-3, C-9, C-2′, C-3′; H_2-2′/C-3, C-1′, C-3′, C-4′; H-3′/C-1′, C-4′, Glc C-1; H_3-4′/C-2′, C-3′; Glc H-1/C-3′; Api H-1/Glc C-6.

3.15. Celephthalide C (**10**)

An amorphous powder, $[\alpha]_D^{23}$ −56° ($c=1.1$, MeOH). Positive FAB-MS *m/z*: 411 [M+K]$^+$, 395 [M+Na]$^+$, 373.1873 [M+H]$^+$ (Calcd for $C_{18}H_{29}O_8$; 373.1862), 211 [M-$C_6H_{10}O_5$+H]$^+$ (base). Negative FAB-MS *m/z*: 371 [M-H]$^-$ (base). For ^1H NMR and ^{13}C NMR spectra, see Tables 3 and 4.

3.16. Acid hydrolysis of **2**, **3**, **5** and **10**

Compounds **2**, **3**, **5** and **10** (5 mg) were each dissolved in 2 N aq. H_2SO_4 and heated on a water bath for 3 h. The hydrolysate was then neutralized with $NaHCO_3$, the salt was filtered off, and the filtrate was purified further over silica gel [$CHCl_3$–MeOH–H_2O (6:4:0.5)] to afford a sugar fraction. From the sugar fraction, D-glucose was detected as described for **1**.

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