FULL PAPER

Seven New Acyl Glycosides from *Erycibe obtusifolia*

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Seven new acyl glycosides, benzyl 5-*O*-vanilloyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 4-hydroxy-3-methoxyphenyl 5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), isopentyl 5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**), 3,4,5-trimethoxyphenyl 5-*O*-sinapoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**4**), 6-methoxy-7-[(6-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy]coumarin (**5**), 6-methoxy-7-[(2-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy]coumarin (**6**), and isopentyl *β*-D-apiofuranosyl-(1→6)-[5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→2)]-*β*-D-glucopyranoside (**7**), were isolated from Chinese folk herb *Erycibe obtusifolia*. Their structures were elucidated on the basis of extensive spectroscopic analysis, including UV, IR, MS, and 1D- and 2D-NMR techniques. Further, these compounds were evaluated against HCT-8 (human colon carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), A549 (human lung carcinoma), and A2780 (human ovarian carcinoma) cell lines, however, none of them exhibited a significant bioactivity (IC_{50} > 10 μM).

Keywords: *Erycibe obtusifolia*, Acyl glycosides, Cytotoxic activity.

Introduction

Erycibe obtusifolia Benth. (Convolvulaceae) is a Chinese folk herb, which is mainly distributed in Asia and Australia. In China, its roots and stems are used to relieve symptoms of rheumatoid arthritis, and to treat some myotic and neural dysfunctions, and other immune-related diseases [1]. Previous chemical investigation revealed that the plant contains various compounds including flavonoids, coumarins, chlorogenic acid derivatives, alkaloids, and monoterpene glycosides [2 – 4]. In our continuing exploration for new bioactive agents from the plant, seven new acyl glycosides were further obtained, including benzyl 5-*O*-vanilloyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 4-hydroxy-3-methoxyphenyl 5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), isopentyl 5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**), 3,4,5-trimethoxyphenyl 5-*O*-sinapoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**4**), 6-methoxy-7-[(6-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy]coumarin (**5**), 6-methoxy-7-[(2-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy]coumarin (**6**), and isopentyl *β*-D-apiofuranosyl-(1→6)-[5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→2)]-*β*-D-glucopyranoside (**7**) (*Fig. 1*), and their structures were determined on the basis of chemical and spectroscopic evidence, including UV, IR, MS, and 1D- and 2D-NMR. Subsequently, the cytotoxicities of these compounds were evaluated against HCT-8 (human colon carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), A549 (human lung carcinoma), and A2780 (human ovarian carcinoma) cell lines.

Results and Discussion

Compound **1** was obtained as white powder, $[\alpha]_D^{20} = -71.3$ (c = 0.05, MeOH). Its molecular formula was determined as $C_{26}H_{32}O_{13}$ based on the pseudomolecular ion in HR-ESI-MS spectrum (m/z 575.1741 ([M + Na]$^+$; calc. 575.1735)). The IR spectrum suggested the presence of OH (3397 cm^{-1}), C=O (1692 cm^{-1}), and benzene groups (1510 cm^{-1}). In the ^{1}H-NMR spectrum, the H-atom signals at δ(H) 7.43 (br. s, 1 H), 6.85 (d, J = 8.0 Hz, 1 H), 7.48 (d, J = 8.0 Hz, 1 H), and 3.78 (s, 3 H) could be attributed to a vanilloyl moiety in comparison with eryciboside D also isolated from this genus [5][6]. Besides, the signals of a monosubstituted benzene ring at δ(H) 7.34 (d, J = 7.5 Hz, 2 H), 7.30 (t, J = 7.5 Hz, 2 H), and 7.25 (t, J = 7.5 Hz, 1 H) together with a pair of geminal coupling H-atom signals at δ(H) 4.74 (d, J = 12.0 Hz, 1 H) and 4.53 (d, J = 12.0 Hz, 1 H) were observed, implying the existence of the benzyloxy moiety. Further, two anomeric H-atoms at δ(H) 4.20 (d, J = 7.5 Hz, 1 H) and 4.97 (br. s, 1 H), and the overlapped signals (δ(H) 3.02 – 4.23) indicated the presence of two sugar moieties. The ^{13}C-NMR spectrum displayed 26 C-atom signals in which the benzyloxy moiety was further confirmed based on the signals at δ(C) 138.5 (C(1)), 128.3 (C(2)/C(6)), 128.7 (C(3)/C

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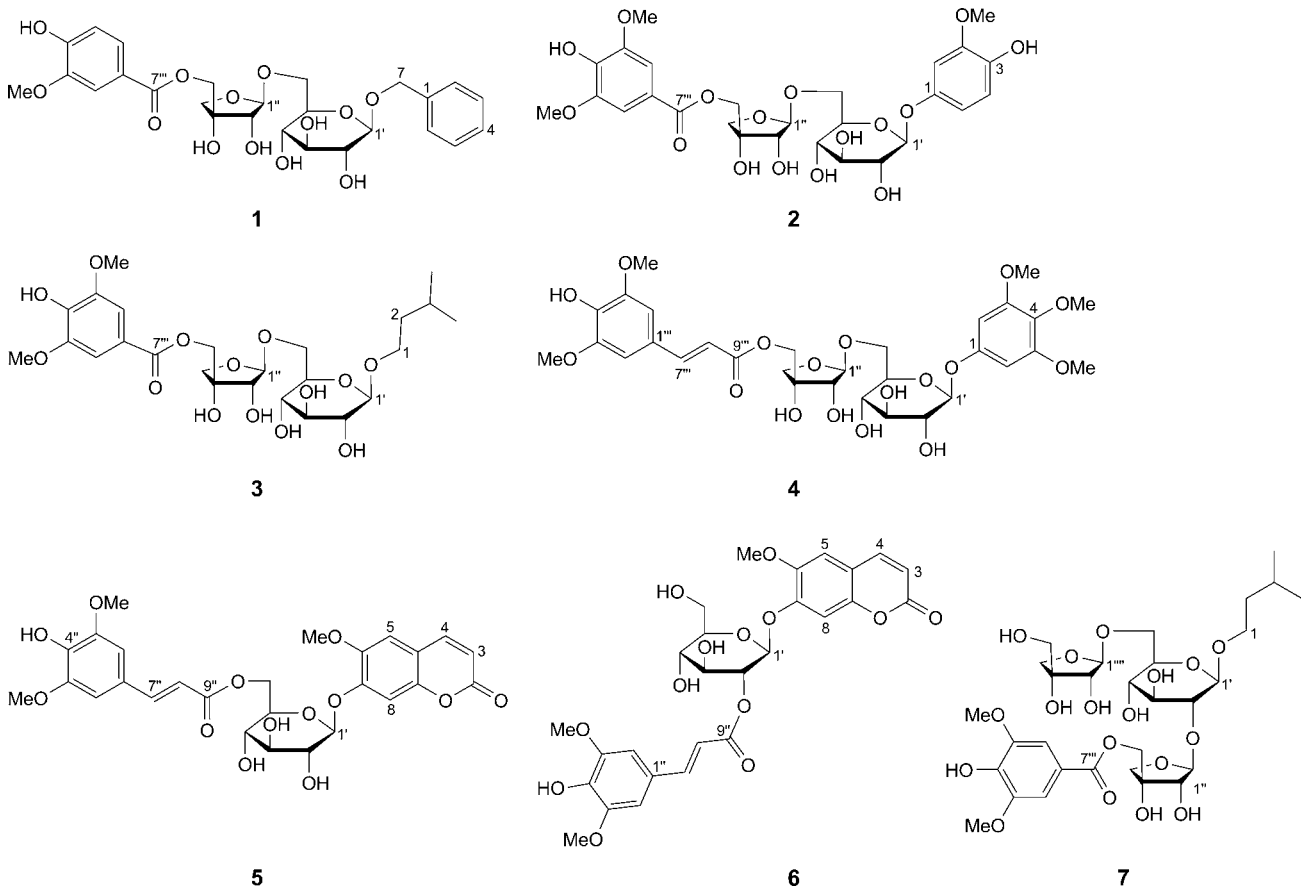

Fig. 1. Structures of isolated compounds **1** – **7**

(5)), 127.9 (C(4)), and 70.1 (C(7)). Also, the vanilloyl moiety was determined according to the resonances at δ(C) 120.8 (C(1‴)), 113.3 (C(2‴)), 147.4 (C(3‴)), 151.8 (C(4‴)), 115.8 (C(5‴)), 124.3 (C(6‴)), 166.1 (C(7‴)), and 56.2 (*Me*O–C(3‴)). The remaining 11 C-atom signals indicated the existence of one hexose and one pentose. D-Apiofuranose and D-glucopyranose were determined by NMR data comparison [7], and gas chromatographic analysis after hydrolysis and derivatization with L-cysteine methyl ester [8]. Furthermore, anomeric chemical shifts and coupling constants (Glc: J = 7.5 Hz; Api: J = br. *s*) confirmed β-configurations [9]. The interglycosidic linkage was established as β-D-apiofuranosyl-(1→6)-β-D-glucopyranose based on the HMBC correlation H–C(1″)/C(6′) (δ(C) 67.7) (*Fig. 2*). In the HMBC spectrum, the correlations H–C(7)/C(1′) and H–C(1′)/C(7) proved the connectivity between benzyl alcohol and glucose, the correlation of H–C(5″)/C(7‴) confirmed the vanilloyl residue to be connected to the position C(5″) of apiofuranosyl (*Fig. 2*). Thus, the structure of compound **1** was determined to be benzyl 5-*O*-vanilloyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** was obtained as white powder, $[\alpha]_D^{20}$ = −78.5 (*c* = 0.05, MeOH). The IR spectrum showed absorptions due to OH (3421 cm^{-1}), C=O (1700 cm^{-1}), and benzene groups (1514 cm^{-1}). The molecular formula $C_{27}H_{34}O_{16}$ was deduced from the positive pseudomolecular ion (*m/z* 637.1735 ([*M* + Na]$^+$; calc. 637.1739)) in the HR-ESI-MS spectrum. Comparing its NMR data with those of obtusifoside H from this plant [2], a 6-*O*-(5-*O*-syringoyl-β-apiofuranosyl)-β-glucopyranosyl moiety could be deduced. In addition, the ^{1}H-NMR spectrum of **2** presented a set of *ABX* system signals (δ(H) 6.61 (*d*, J = 2.5 Hz, 1 H), 6.63 (*d*, J = 8.5 Hz, 1 H), 6.45 (*dd*, J = 8.5, 2.5 Hz, 1 H)) and an additional MeO resonance at δ(H) 3.70 (*s*, 3 H). Further, the ^{13}C-NMR signals confirmed the aforementioned benzene ring moiety and the MeO signal (δ(C) 55.5). According to the above information, the aglycone was determined to be 4-hydroxy-3-methoxyphenyl [10]. The HMBC correlations (*Fig. 2*) from H–C(1″) to C(6′) and from H–C(5″) to C(7‴) confirmed the connectivities between the syringoyl, apiofuranosyl, and glucopyranosyl moieties. The correlations from H–C(1′) to C(1) and from H–C(2) to C(1′) proved the sugar chain to be attached to position C(1) of the benzene ring. The D-configurations of the sugars were determined by the same method as for compound **1**. Therefore, the structure of compound **2** was elucidated as 4-hydroxy-3-methoxyphenyl 5-*O*-syringoyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

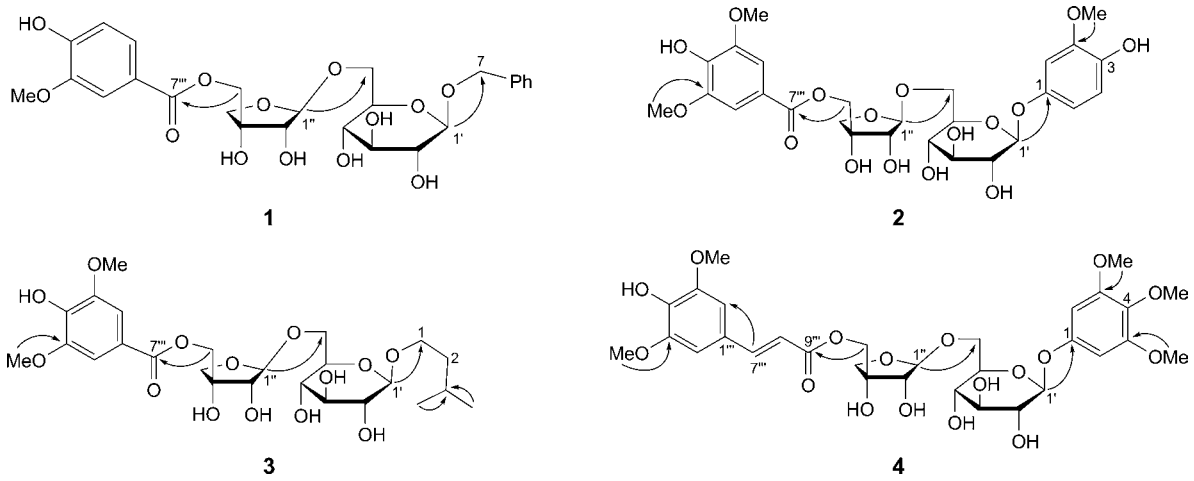

Fig. 2. Selected HMBC correlations of compound **1** – **4**

Compound **3** was obtained as white powder, $[\alpha]_D^{20}$ = −67.9 (*c* = 0.05, MeOH). The IR spectrum suggested the existence of OH (3413 cm^{-1}), C=O (1708 cm^{-1}), and benzene groups (1516 cm^{-1}). The HR-ESI-MS gave the molecular formula $C_{25}H_{38}O_{14}$ (*m/z* 585.2158 ($[M + Na]^+$); calc. 585.2154)). Similar to compound **2**, the 6-*O*-(5-*O*-syringoyl-*β*-apiofuranosyl)-*β*-glucopyranosyl moiety was also readily deduced by the ^{1}H- and ^{13}C-NMR spectrum (*Table 1*). The remaining H-atom signals included two oxygenated CH_2 groups at *δ*(H) 3.85 and 3.43, two CH_2 group at *δ*(H) 1.33 (*m*, H–C(2), 2 H), a CH proton at *δ*(H) 1.58 – 1.64 (*m*, H–C(3), 1 H), and two Me groups at *δ*(H) 0.81 (*d*, *J* = 6.5 Hz, H–C(4), 3 H) and 0.80 (*d*, *J* = 6.5 Hz, H–C(5), 3 H). Taking also into the consideration that the remaining five ^{13}C-NMR signals at *δ*(C) 67.7 (C(1)), 38.0 (C(2)), 24.2 (C(3)), 22.4 (C(4)), and 22.5 (C(5)), the aglycone of compound **3** was confirmed as isoamyl alcohol. In the HMBC experiment, the correlations of H–C(1) with C(1′), H–C(1′) with C(1), H–C(6′) with C(1″), and H–C(5″) with C(7‴) confirmed the connectivities between these moieties (*Fig. 2*). The D-configurations of the sugars were determined by the same method as for compound **1**. Therefore, the structure of compound **3** was determined to be isopentyl 5-*O*-syringoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **4** was obtained as white powder, $[\alpha]_D^{20}$ = −76.1 (*c* = 0.05, MeOH), the maximum absorptions of UV spectrum were at 260 and 330 nm. The IR spectrum gave the absorptions of OH (3418 cm^{-1}), C=O (1695 cm^{-1}), and benzene groups (1507 cm^{-1}). The molecular formula was determined to be $C_{31}H_{40}O_{17}$ by the HR-ESI-MS (*m/z* 707.2168 ($[M + Na]^+$; calc. 707.2158)). When comparing with obtusifoside E [2], the ^{1}H- and ^{13}C-NMR data (*Table 1*) showed the signals corresponding to 6-*O*-(5-*O*-sinapoyl-*β*-apiofuranosyl)-*β*-glucopyranosyl. Additionally, a pair of aromatic protons (*δ*(H) 7.02 (*s*, H–C(2)/(6), 2 H)) and three MeO signals (*δ*(H) 3.72 (*s*, MeO–C(3)/(5), 6 H), 3.56 (*s*, MeO–C(4), 3 H)) were observed in the ^{1}H-NMR spectrum. The benzene ring C-atom signals at *δ*(C) 153.9 (C(1)), 94.3 (C(2/6)), 153.1 (C(3/5)), and 132.5 (C(4)), as well as three MeO signals at *δ*(C) 55.7 (MeO–C(3)/(5)) and 60.0 (MeO–C(4)) were presented in the ^{13}C-NMR spectrum. According to the above information, the aglycone of compound **4** was confirmed to be 3,4,5-trimethoxyphenyl. The HMBC correlations H–C(1′)/C(1), H–C(1″)/C(6′), and H–C(5″)/C(9‴) (*Fig. 2*) confirmed the connectivity between these moieties. The D-configurations of the sugars were determined by the same method as for compound **1**. Therefore, the structure of compound **4** was identified as 3,4,5-trimethoxyphenyl 5-*O*-sinapoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **5** was obtained as yellow powder, $[\alpha]_D^{20}$ = −94.9 (*c* = 0.05, MeOH). In the UV spectrum, absorption maxima were observed at 280 and 340 nm. The IR spectrum suggested the presence of OH (3386 cm^{-1}), C=O (1702 cm^{-1}), and benzene ring (1515 cm^{-1}). The HR-ESI-MS indicated the molecular formula to be $C_{27}H_{28}O_{13}$ on the basis of pseudomolecular ion peak (*m/z* 561.1612 ($[M + H]^+$; calc. 561.1603)). The ^{1}H-NMR (*Table 2*) spectrum showed characteristic coumarin resonances at *δ*(H) 6.23 (*d*, *J* = 9.5 Hz, 1 H) and 7.90 (*d*, *J* = 9.5 Hz, 1 H), while two aromatic singlets at *δ*(H) 7.27 (*s*, 1 H) and 7.16 (*s*, 1 H) suggested that the oxygen-substituted positions were at C(6) and C(7). In addition, ^{1}H- and ^{13}C-NMR data (*Table 2*) displayed the resonances at *δ*(H) 6.91 (*s*, H–C(2″)/(6″)), 6.43 (*d*, *J* = 16.0 Hz, H–C(7″)), 7.47 (*d*, *J* = 16.0 Hz, H–C(8″)), and 3.77 (*s*, MeO–C(3″)/(5″)); *δ*(C) 124.1 (C(1″)), 106.2 (C(2″/6″)), 148.0 (C(3″/5″)), 138.5 (C(4″)), 145.7 (C(7″)), 114.3 (C(8″)), and 166.5 (C(9″)), which were corresponding to a sinapoyl moiety. The other ^{1}H-NMR data at *δ*(H) 3.08 – 5.28 including the anomeric H-atom signals at *δ*(H) 5.19 (*d*, *J* = 7.0 Hz, H–C(1′)), as well as the ^{13}C-NMR signals at *δ*(C) 99.2 (C(1′)), 73.0 (C(2′)), 76.4 (C(3′)), 70.0 (C(4′)), 73.7 (C(5′)), and 63.1 (C(6′)) implied that **5** contained a glucose fragment. The D-configuration of the glucose was determined by the same method as for compound **1**. In the HMBC experiment (*Fig. 3*), the correlation of H–

Table 1. ^{1}H- and ^{13}C-NMR Data (500 and 125 MHz, resp.; (D_6)DMSO) of **1** – **4**. δ in ppm, J in Hz

Position	**1**		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		138.5		150.7	3.85 – 3.86 (*m*), 3.43 – 3.44 (*m*)	67.7		153.9
2	7.34 (*d*, *J* = 7.5)	128.3	6.61 (*d*, *J* = 2.5)	102.4	1.30 – 1.33 (*m*), 1.33 – 1.36 (*m*)	38.0	6.33 (*s*)	94.3
3	7.30 (*t*, *J* = 7.5)	128.7		147.8	1.58 – 1.64 (*m*)	24.2		153.1
4	7.25 (*t*, *J* = 7.5)	127.9		141.5	0.81 (*d*, *J* = 6.5)	22.4		132.5
5	7.30 (*t*, *J* = 7.5)	128.7	6.63 (*d*, *J* = 8.5)	115.3	0.80 (*d*, *J* = 6.5)	22.5		153.1
6	7.34 (*d*, *J* = 7.5)	128.3	6.45 (*d*, *J* = 8.5, 2.5)	107.9			6.33 (*s*)	94.3
7	4.74 (*d*, *J* = 12.0), 4.53 (*d*, *J* = 12.0)	70.1						
1′	4.20 (*d*, *J* = 7.5)	102.5	5.12 (*d*, *J* = 9.0)	101.6	4.06 (*d*, *J* = 7.5)	102.7	4.80 (*d*, *J* = 7.5)	100.8
2′	3.01 (overlap)	74.0	3.18 (*t*, *J* = 9.0)	73.2	2.90 – 2.92 (*m*)	73.3	3.20 (*t*, *J* = 9.0)	73.1
3′	3.12 (*t*, *J* = 8.5)	77.2	3.24 (*t*, *J* = 9.0)	76.5	3.07 – 3.11 (*m*)	76.7	3.27 (*t*, *J* = 9.0)	76.4
4′	3.02 (overlap)	70.9	3.07 (*t*, *J* = 9.5)	70.0	2.95 – 2.97 (*m*)	70.3	3.08 (*t*, *J* = 9.0)	70.0
5′	3.25 – 3.26 (*m*)	76.2	3.42 – 3.43 (*m*)	75.4	3.24 (*t*, *J* = 8.5)	75.4	3.50 – 3.51 (*m*)	75.4
6′	3.92 (*d*, *J* = 9.5), 3.44 – 3.45 (*m*)	67.7	3.91 (*d*, *J* = 9.5), 3.44 – 3.45 (*m*)	67.7	3.67 – 3.69 (*m*), 3.38 – 3.39 (*m*)	66.8	3.92 (*t*, *J* = 10.0), 3.41 – 3.43 (*m*)	67.7
1″	4.97 (br. *s*)	109.6	4.89 (br. *s*)	108.9	4.92 (br. *s*)	109.0	4.86 (br. *s*)	108.5
2″	3.83 – 3.85 (*m*)	77.4	3.80 (overlap)	77.1	3.81 (overlap)	76.8	3.70 (overlap)	76.6
3″		77.7		77.1		77.1		77.0
4″	3.95 (*d*, *J* = 9.5), 3.75 (*d*, *J* = 9.5)	74.0	3.94 (*d*, *J* = 9.5), 3.77 (*d*, *J* = 9.5)	73.4	3.93 (*d*, *J* = 9.5), 3.77 (*d*, *J* = 9.5)	73.3	3.90 (*d*, *J* = 9.5), 3.72 (*d*, *J* = 9.5)	73.3
5″	4.23 (br. *s*)	67.0	4.23 (br. *s*)	66.6	4.22 (br. *s*)	66.7	4.11 (br. *s*)	65.7
1‴		120.8		119.7		119.0		124.1
2‴	7.43 (br. *s*)	113.3	7.24 (*s*)	107.1	7.22 (*s*)	107.1	7.02 (*s*)	106.3
3‴		147.4		147.8		147.5		148.0
4‴		151.8		141.3		140.1		138.1
5‴	6.85 (*d*, *J* = 8.0)	115.8		147.8		147.5		148.0
6‴	7.48 (*d*, *J* = 8.0)	124.3	7.24 (*s*)	107.1	7.22 (*s*)	107.1	7.02 (*s*)	106.3
7‴		166.1		165.8		166.5	7.57 (*d*, *J* = 16.0)	145.7
8‴							6.52 (*d*, *J* = 16.0)	114.6
9‴								166.5
*Me*O–C(3,5)			3.70 (*s*)	55.5			3.72 (*s*)	55.7
*Me*O–C(4)							3.56 (*s*)	60.0
*Me*O–C(3‴)	3.78 (*s*)	56.2	3.80 (*s*)	56.2	3.79 (*s*)	56.1	3.79 (*s*)	56.1
*Me*O–C(5‴)			3.80 (*s*)	56.2	3.79 (*s*)	56.1	3.79 (*s*)	56.1

C(6′)/C(9″) confirmed the sinapoyl to be connected with C(6′) of the glucose moiety. The correlations of *Me*O–C (6) (δ(H) 3.80 (*s*, 3 H))/C(6), and H–C(1′)/C(7) indicated that the MeO group and glucose were connected on the positions C(6) and C(7) of the coumarin, respectively. Thus, compound **5** was elucidated to be 6-methoxy-7-[(6-*O*-sinapoyl-β-D-glucopyranosyl)oxy]coumarin.

Compound **6** was obtained as yellow powder, $[\alpha]_D^{20}$ = −40.8 (*c* = 0.05, MeOH). The molecular formula $C_{27}H_{28}O_{13}$ was determined by the HR-ESI-MS (*m/z* 561.1602 ($[M + H]^+$; calc. 561.1603)). Similar to compound **5**, the ^{1}H- and ^{13}C-NMR (*Table 2*) also showed the signals of coumarin, glucose, and sinapoyl. Careful comparison of the ^{13}C-NMR data with those of compound **5** revealed that the resonances of H–C(2′) ($\Delta\delta$(H) +1.62) and C(6′) ($\Delta\delta$(C) −2.5) were shifted in compound **6**, indicating that the attachment position of glucose in **6** was not on position C(6′) but on C(2′) of the glucose. The HMBC experiment (*Fig. 3*) also supported this conclusion by the correlations of H–C(2′)/C(9″). Additional correlations C(6)/*Me*O–C(6) (δ(H) 3.67 (3 H, *s*)) and C(7)/H–C(1′) confirmed that the MeO group and sugar chain were located at the positions C(6) and C(7) of coumarin, respectively. Therefore, compound **6** was determined to be 6-methoxy-7-[(2-*O*-sinapoyl-β-D-glucopyranosyl)oxy]coumarin.

Compound **7** was obtained as white powder, $[\alpha]_D^{20}$ = −53.9 (*c* = 0.05, MeOH). In the UV spectrum, absorption maximum was observed at 278 nm. The IR spectrum showed the absorptions of OH (3393 cm^{-1}), C=O (1692 cm^{-1}), and benzene group (1512 cm^{-1}). The HR-ESI-MS gave the pseudomolecular ion (*m/z* 717.2585 ($[M + Na]^+$; calc. 717.2576)), corresponding to the molecular formula $C_{30}H_{46}O_{18}$. Comparing the NMR data with those of 1-*O*-[2,6-*O*-bis(5-*O*-syringoyl-β-D-apiofuranosyl)-β-D-glucopyranosyl]isoamyl alcohol [11], compound **7** also showed two sets of apiofuranosyl signals and a set of glucose signals (*Table 3*). The D-configurations of the sugars were determined by the same method as for compound **1**. The other H-atom signals at δ(H) 3.66 – 3.67 (*m*, H–C(1)), 3.21 – 3.22 (*m*, H–C(1)), 1.21 – 1.35 (2 *m*, H–C(2)), 1.46 –

Table 2. ^{1}H- and ^{13}C-NMR Data (500 and 125 MHz, resp.; (D_6)DMSO) of **5** and **6**. δ in ppm, J in Hz

Position	**5**		**6**	
	δ(H)	δ(C)	δ(H)	δ(C)
2		160.3		160.3
3	6.23 (*d*, *J* = 9.5)	113.3	6.33 (*d*, *J* = 9.5)	113.9
4	7.90 (*d*, *J* = 9.5)	144.0	7.93 (*d*, *J* = 9.5)	144.1
5	7.27 (*s*)	109.7	7.25 (*s*)	110.4
6		145.9		146.3
7		149.5		149.5
8	7.16 (*s*)	102.9	7.24 (*s*)	104.5
9		148.8		148.8
10		112.3		113.2
1′	5.19 (*d*, *J* = 7.0)	99.2	5.28 (*d*, *J* = 8.0)	98.5
2′	3.33 (overlap)	73.0	4.95 (*t*, *J* = 8.0)	73.1
3′	3.33 (overlap)	76.4	3.52 – 3.54 (*m*)	77.5
4′	3.26 – 3.28 (*m*)	70.0	3.26 – 3.30 (*m*)	70.0
5′	3.77 (overlap)	73.7	3.54 – 3.56 (*m*)	74.2
6′	4.38 (*d*, *J* = 11.0)	63.1	3.74 – 3.76 (*m*),	60.6
	4.18 – 4.25 (*m*)		3.50 – 3.51 (*m*)	
1″		124.1		124.3
2″	6.91 (*s*)	106.2	7.00 (*s*)	106.2
3″		148.0		148.1
4″		138.5		138.5
5″		148.0		148.1
6″	6.91 (*s*)	106.2	7.00 (*s*)	106.2
7″	7.47 (*d*, *J* = 16.0)	145.7	7.55 (*d*, *J* = 16.0)	145.5
8″	6.43 (*d*, *J* = 16.0)	114.3	6.52 (*d*, *J* = 16.0)	115.0
9″		166.5		165.6
*Me*O–C(6)	3.80 (*s*)	56.0	3.67 (*s*)	56.0
*Me*O–C(3″,5″)	3.77 (*s*)	56.0	3.77 (*s*)	56.4

1.54 (*m*, H–C(3)), 0.71 (*d*, *J* = 5.0 Hz, MeO–C(4)), 0.70 (*d*, *J* = 5.0 Hz, H–C(5)), and C-atom signals at δ(C) 67.0 (C(1)), 38.1 (C(2)), 24.5 (C(3)), 22.5 (C(4)), 22.3 (C(5)) were attributed to an isoamyloxy residue. The comparison indicated that only one syringoyl moiety was present in compound **7**. The HMBC correlations (*Fig. 3*) H–C(5″)/C(7‴) and H–C(1″)/C(2′) proved that the 5-*O*-syringoyl-β-D-apiofuranosyl moiety was located on the position C(2′) of the glucose. The correlation of H–C(1‴)/C(6′) proved a β-D-apiofuranosyl connected on position C(6′) of the glucose moiety. At the same time, the correlations H–C(1′)/C(1) and H–C(1)/C(1′) confirmed the connectivity between glucose and isoamyl alcohol. Therefore, the structure of compound **7** was identified as isopentyl β-D-apiofuranosyl-(1→6)-[5-*O*-syringoyl-β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside.

The cytotoxicities of these compounds were evaluated against the A549, Bel7402, BGC-823, HCT-8, and A2780 cell lines. However, they were inactive against these tumor cell lines (IC_{50} > 10 μM).

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Experimental Part

General

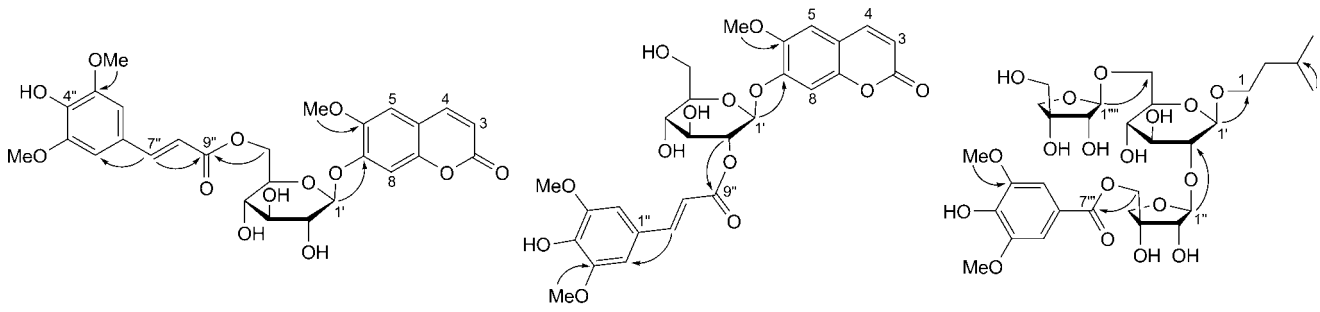
Column chromatography (CC): macroporous resin (*Diaion HP-20*, *Mitsubishi Chemical Corp.*, Tokyo, Japan), *Rp-18* (50 μm, *YMC*, Kyoto, Japan), *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Uppsala, Sweden), silica gel (200 – 300 mesh, *Qingdao Marine Chemical Inc.* Qingdao, P. R. China). Prep. HPLC: *Shimadzu LC-6AD* instrument with an *SPD-20A* detector; *YMC-Pack ODS-A* column (250 × 20 mm, 5 μm). HPLC-DAD Analysis: *Agilent 1260 series* system (*Agilent Technologies*, Waldbronn, Germany); *Apollo C_{18}* column (250 × 4.6 mm, 5 μm, *Grace Davison*, IL, USA); D-apiose (*International Laboratory*, San Francisco, CA, USA), D-glucose (J&K, Beijing, China). Optical rotations: *JASCO P-2000* polarimeter. UV Spectra: *JASCO V-650* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet 5700* spectrometer by a FT-IR microscope transmission method; $\tilde{\nu}$ in cm^{-1}. GC: *Agilent 7890A* instrument. 1D- and 2D-NMR spectra: *INOVA 500* spectrometers. HR-ESI-MS: *Agilent 1100 series LC/MSD ion trap mass* spectrometer; in *m*/*z*. ESI-MS: *Agilent 1100 series LC/MSD TOF* (*Agilent Technologies*); in *m*/*z*.

Plant Material

The stems of *Erycibe obtusifolia* Benth. were collected in Jianfengling National Nature Reserve of Hainan Province, China, in March 2010. The plant material was identified by Prof. *Huanqiang Chen* (Jianfengling National Nature Reserve of Hainan Province). A voucher specimen (ID-21180) was deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China.

Extraction and Isolation

The dried stems of *Erycibe obtusifolia* Benth. (18.5 kg) were extracted with 95% EtOH (3 × 30 l, 1.5 h each) under reflux. The extract was concentrated under reduced pressure to give a residue (0.96 kg), which was suspended in H_2O (2.3 l) and sequentially partitioned with petroleum ether (3 × 2.0 l), AcOEt (3 × 2.0 l), and *n*-BuOH (3 × 2.0 l). After the solvent was evaporated under reduced pressure, the *n*-BuOH extract (400 g) was subjected to CC (*macroporous resin HP-20*; EtOH/H_2O 0:100→95:5). After removing the solvent, the 50% EtOH fraction (50 g) was subjected to CC (*Sephadex LH-20*; MeOH/H_2O 1:10→100:0) to obtain 10 fractions (A_1 – A_{10}) through a HPLC-DAD analysis. Fraction A_1 (500 mg) was subjected to preparative HPLC (*YMC-Pack ODS*; MeOH/H_2O 40:60; flow rate 4 ml/min) to give **4** (23 mg; t_R = 39 min), **5** (16 mg; t_R = 41 min), and **6** (17 mg; t_R = 35 min). Fraction A_5 (300 mg) was further separated by preparative HPLC (*YMC-Pack ODS*; MeOH/H_2O 45:55; flow rate 4 ml/min) to obtain **1** (15 mg; t_R = 45 min), **2**



5 **6** **7**

Fig. 3. Selected HMBC correlations of compound **5** – **7**

Table 3. ^{1}H- and ^{13}C-NMR Data (500 and 125 MHz, resp.; (D_6)DMSO) of **7**. δ in ppm, J in Hz

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	3.66 – 3.67 (*m*), 3.21 – 3.22 (*m*)	67.0	5″	4.31 (*d*, *J* = 11.0), 4.19 (*d*, *J* = 11.0)	67.9
2	1.21 – 1.35 (*m*)	38.1	1‴		119.2
3	1.46 – 1.54 (*m*)	24.5	2‴	7.23 (*s*)	107.3
4	0.71 (*d*, *J* = 5.0)	22.5	3‴		147.7
5	0.70 (*d*, *J* = 5.0)	22.3	4‴		141.2
1′	4.14 (*d*, *J* = 8.0)	101.1	5‴		147.7
2′	3.22 (overlap)	75.4	6‴	7.23 (*s*)	107.3
3′	3.29 – 3.30 (*m*)	77.1	7‴		165.6
4′	3.17 – 3.19 (*m*)	70.6	*Me*O–C(3‴,5‴)	3.78 (*s*)	56.2
5′	3.62 – 3.64 (*m*)	75.1	1⁗	4.84 (br. *s*)	109.2
6′	3.80 – 3.82 (*m*), 3.37 – 3.40 (*m*)	67.5	2⁗	3.72 – 3.74 (*m*)	75.9
			3⁗		78.8
1″	5.34 (br. *s*)	108.1	4⁗	3.82 (*d*, *J* = 9.0), 3.56 (*d*, *J* = 9.0)	73.4
2″	3.78 (overlap)	76.7			
3″		77.5	5⁗	3.26 – 3.28 (*m*), 3.32 – 3.35 (*m*)	63.2
4″	4.03 (*d*, *J* = 9.5), 3.81 (*d*, *J* = 9.5)	73.9			

(50 mg; t_R = 46 min), and **3** (18 mg; t_R = 48 min). The 30% EtOH fraction (200 g) was also applied to CC (*Sephadex LH-20*; MeOH/H_2O 1:10→100:0) to yield 20 fractions (B_1 – B_{20}) through a HPLC-DAD analysis. Fraction B_{12} (3 g) was subjected to CC (*Sephadex LH-20*; MeOH/H_2O 0:100→100:0), resulting in four subfractions ($B_{12\text{-}1}$ – $B_{12\text{-}4}$). Using the mobile phase (MeOH/H_2O 45:55), subfraction $B_{12\text{-}3}$ was separated by preparative HPLC (*YMC-Pack ODS;* MeOH/H_2O 35:65; flow rate 4 ml/min) to yield **7** (25 mg; t_R = 45 min).

Benzyl 5-*O*-Vanilloyl-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (= **Benzyl 5-*O*-(4-Hydroxy-3-methoxybenzoyl)-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside**; **1**). White powder. $[\alpha]_D^{20}$ = –71.3 (*c* = 0.05, MeOH). UV (MeOH): 260 (4.08), 290 (4.02). IR: 3397, 1692, 1595, 1510, 1342, 1283, 1109, 827, 766. ^{1}H- and ^{13}C-NMR ((D_6) DMSO): *Table 1*. HR-ESI-MS (pos.): 575.1741 ([*M* + Na]$^+$, $C_{26}H_{32}NaO_{13}^+$; calc. 575.1735).

4-Hydroxy-3-methoxyphenyl 5-*O*-Syringoyl-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (= **4-Hydroxy-3-methoxyphenyl 5-*O*-(4-Hydroxy-3,5-dimethoxybenzoyl)-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside**; **2**). White powder. $[\alpha]_D^{20}$ = –78.5 (*c* = 0.05, MeOH). UV (MeOH): 260 (4.06), 280 (4.16). IR: 3421, 1700, 1615, 1514, 1462, 1336, 1229, 1115, 1069, 802, 764. ^{1}H- and ^{13}C-NMR ((D_6) DMSO): *Table 1*. ESI-MS (pos.): 614 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 637.1735 ([*M* + Na]$^+$, $C_{27}H_{34}NaO_{16}^+$; calc. 637.1739).

Isopentyl 5-*O*-Syringoyl-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (= **3-Methylbutyl 5-*O*-(4-Hydroxy-3,5-dimethoxybenzoyl)-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside**; **3**). White powder. $[\alpha]_D^{20}$ = –67.9 (*c* = 0.05, MeOH). UV (MeOH): 277 (4.07). IR: 3413, 1708, 1612, 1516, 1463, 1336, 1220, 1115, 764. ^{1}H- and ^{13}C-NMR ((D_6)DMSO): *Table 1*. HR-ESI-MS (pos.): 585.2158 ([*M* + Na]$^+$, $C_{25}H_{38}NaO_{14}^+$; calc. 585.2154).

3,4,5-Trimethoxyphenyl 5-*O*-Sinapoyl-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (= **3,4,5-Trimethoxyphenyl 5-*O*-[(2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside**; **4**). Yellow powder. $[\alpha]_D^{20}$ = –76.1 (*c* = 0.05, MeOH). UV (MeOH): 260 (4.06), 330 (3.85). IR: 3418, 1695, 1602, 1507, 1461, 1256, 1120, 832. ^{1}H- and ^{13}C-NMR ((D_6) DMSO): *Table 1*. ESI-MS (pos.): 707 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 707.2168 ([*M* + Na]$^+$, $C_{31}H_{40}NaO_{17}^+$; calc. 707.2158).

6-Methoxy-7-[(6-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy] coumarin (= **7-({6-*O*-[(2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-*β*-D-glucopyranosyl}oxy)-6-methoxy-2*H*-1-benzopyran-2-one**; **5**). Yellow powder. $[\alpha]_D^{20}$ = –94.9 (*c* = 0.05, MeOH). UV (MeOH): 280, 340. UV (MeOH): 280 (4.22), 340 (4.75). IR: 3386, 1702, 1616, 1567, 1515, 1461, 1281, 1116, 826. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 2*. ESI-MS (pos.): 561 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 561.1612 ([*M* + H]$^+$, $C_{27}H_{29}O_{13}^+$; calc. 561.1603).

6-Methoxy-7-[(2-*O*-sinapoyl-*β*-D-glucopyranosyl)oxy] coumarin (= **7-({2-*O*-[(2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-*β*-D-glucopyranosyl}oxy)-6-methoxy-2*H*-1-benzopyran-2-one**; **6**). Yellow powder. $[\alpha]_D^{20}$ = –40.8 (*c* = 0.05, MeOH). UV (MeOH): 280, 340. UV (MeOH): 280 (4.25), 340 (4.76). IR: 3390, 1712, 1611, 1565, 1514, 1460, 1280, 1118, 827. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 2*. ESI-MS (pos.): 561 ([*M* + H]$^+$). HR-ESI-MS (pos.): 561.1602 ([*M* + H]$^+$, $C_{27}H_{29}O_{13}^+$; calc. 561.1603).

Isopentyl *β*-D-Apiofuranosyl-(1 → 6)-[5-*O*-syringoyl-*β*-D-apiofuranosyl-(1 → 2)]-*β*-D-glucopyranoside (= **3-Methylbutyl *β*-D-Apiofuranosyl-(1 → 6)-[5-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-*β*-D-apiofuranosyl-(1 → 2)]-*β*-D-galactopyranoside**; **7**). White powder. $[\alpha]_D^{20}$ = –53.9 (*c* = 0.05, MeOH). UV (MeOH): 278 (4.09). IR: 3393, 2953, 1692, 1594, 1512, 1464, 1286, 1224, 764. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 3*. HR-ESI-MS (pos.): 717.2585 ([*M* + Na]$^+$, $C_{30}H_{46}NaO_{18}^+$; calc. 717.2576).

Acid Hydrolysis and Sugar Analysis

The procedure previously reported was used [11], compounds **1** – **7** (2 mg each) were dissolved in 1M HCl 5 ml and heated at 60 °C for 2 h in a H_2O bath, respectively. The mixture was concentrated under reduced pressure. The residue was suspended in H_2O and extracted with EtOAc. The aqueous layer was evaporated, repeatedly diluted with H_2O and evaporated to give a neutral residue. The residue was dissolved in anhydrous pyridine (1 ml). L-Cysteine methyl ester hydrochloride (2 mg) was then added and the mixture was incubated at 60 °C for 1 h. After evaporation to dryness, 0.5 ml of *N*-trimethylsilylimidazole was added, and the mixture was further incubated at 60 °C for 1 h. The products were partitioned between *n*-hexane and H_2O (2 ml each). The *n*-hexane extract was subjected to GC analysis on a capillary *HP-5* column, (60 m × 0.25 mm, with a 0.25 μm film, *Dikma*); detection, FID; detector temperature, 280 °C; injection temperature, 260 °C; initial temperature 160 °C, raised to 280 °C at 5 °C/min and final temperature maintained for 10 min; and carrier N_2 gas. D-Glucose and D-apiose were confirmed in the hydrolysates by comparing their retention times with those of authentic sugars derivatized following the same procedure.

Cytotoxicity Assay

Compounds **1** – **7** were tested for cytotoxicity against HCT-8 (human colon carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), A549 (human lung carcinoma), and A2780 (human ovarian carcinoma) by means of an MTT method described in the literature [12].

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