Itosides A – I, New Phenolic Glycosides from *Itoa orientalis*

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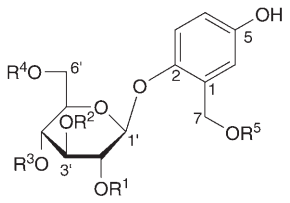
Eight new benzoylated gentisyl alcohol (=2-(hydroxymethyl)benzene-1,4-diol) glucosides, itosides A – H (**1** – **8**), together with the new pyrocatechol (=benzene-1,2-diol) glycoside itoside I (**9**) were isolated from the bark and twigs of *Itoa orientalis* (Flacourtiaceae). In itosides B – D (**2** – **4**), the gentisyl alcohol moiety was esterified by 1-hydroxy-6-oxocyclohex-2-ene-1-carboxylic acid, while itosides E – H (**5** – **8**) contained instead an additional 2-hydroxybenzoic acid moiety. The compounds were accompanied by the known derivatives 4-hydroxytremulacin (**10**), poliothyrsoside (**11**), poliothyrsin (**12**), homaloside D (**13**), tremulacin, and pyrocatechol β-D-glucopyranoside. The structures of the new compounds were elucidated by spectral and chemical methods.

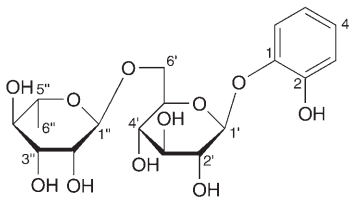
1. Introduction. – *Itoa orientalis* HEMSL. (Flacourtiaceae) is mainly distributed in the southwest of Guangxi Province of China. Its barks and roots, named 'Dahuangshu' in Chinese, have been used in folk medicine for the treatment of rheumatism, injuries from falls, hepatitis, and anaemia [1]. However, there were no reports on constituents of *I. orientalis* and other *Itoa* species. As part of our systematic investigation of Flacourtiaceae plants in China [2], the constituents of *I. orientalis* were investigated. In this paper, we describe the isolation and structure elucidation of nine new phenolic glycosides which were named itosides A – I (**1** – **9**) from the barks and twigs, together with six known glycosides, *i.e.*, 4-hydroxytremulacin (**10**) [3], poliothyrsoside[1]) (**11**) [4], poliothyrsin (**12**) [4], homaloside D (**13**) [5], tremulacin (=2-{{[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl}phenyl β-D-glucopyranoside 2-benzoate) [3][6], and pyrocatechol β-D-glucopyranoside (=2-hydroxyphenyl β-D-glucopyranoside) [7]. The structures of compounds **1** – **9** were elucidated by spectral and chemical methods, while the known compounds were identified by comparing their NMR data with those reported.

2. Results and Discussion. – Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{20}H_{22}O_9$ by the pseudo-molecular-ion peak at *m/z* 429.1156 ($[M + Na]^+$) in the HR-ESI-MS. The IR spectrum showed absorption bands due to OH (3434 cm^{-1}) and C=O (1716 cm^{-1}) groups. D-Glucose was detected by TLC and GC analyses after acid hydrolysis. Compound **1** was

[1]) Named 'polio**thry**soside' in [4].



	R^1	R^2	R^3	R^4	R^5
1	COPh	H	H	H	H
11	H	H	H	COPh	H
2	COPh	H	H	COPh	A
10	COPh	H	H	H	A
3	H	COPh	H	H	A
4	H	H	COPh	H	A
12	H	H	H	H	A
13	H	H	H	COPh	A
5	COPh	H	H	H	B
6	H	COPh	H	H	B
7	H	H	COPh	H	B
8	H	H	H	COPh	B



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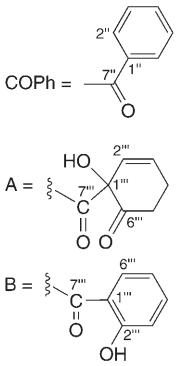

elucidated as 4-hydroxy-2-(hydroxymethyl)phenyl β-D-glucopyranoside 2-benzoate and named itoside A.

The ^{1}H-NMR spectrum of **1** (*Table 1*) revealed a benzoyl moiety (δ 7.48 (*t*, $J = 8.0$ Hz, 2 H), 7.60 (*t*, $J = 8.0$ Hz, 1 H), and 8.08 (*d*, $J = 8.0$ Hz, 2 H)), and an *ABX* system (δ 6.98 (*d*, $J = 8.5$ Hz, 1 H), 6.58 (*dd*, $J = 8.5$, 3.0 Hz, 1 H) and 6.77 (*d*, $J = 3.0$ Hz, 1 H)) suggesting a structure similar to that of poliothyrsoside (=4-hydroxy-2-(hydroxymethyl)phenyl β-D-glucopyranoside 6-benzoate; **11**) [4]. Comparison of the ^{13}C-NMR spectra of **1** (*Table 2*) with those of **11** showed a downfield shift for C(2′) and upfield shifts for C(1′) and C(3′), thus indicating that in **1**, the benzoyl group was attached to OC(2′). The above assignments were confirmed by 2D NMR spectra (^{1}H,^{1}H-COSY and HMBC). In addition, the HMBC plot showed the correlations δ(H) 5.05 (H–C(1′))/δ(C)148.9 (C(2)) and δ(H) 5.22 (H–C(2′))/δ(C) 167.3 (C(7″)).

Compound **2** was obtained as a colorless syrup. Its molecular formula was established as $C_{34}H_{32}O_{13}$ by the pseudo-molecular ion-peak at *m/z* 671.1714 ($[M + Na]^+$) in the HR-ESI-MS. The IR spectrum showed absorption bands due to OH (3425 cm^{-1}) and C=O (1721 cm^{-1}) groups. Compound **2** was elucidated as 4-hydroxy-2-{{[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl}phenyl β-D-glucopyranoside 2,6-dibenzoate and named itoside B.

The ^{1}H-NMR spectrum of **2** (*Table 3*) showed two benzoyl moieties (δ 7.43–7.50 (*m*, 4 H), 7.52–7.59 (*m*, 2 H), 7.98 (*dd*, $J = 8.0$, 1.5 Hz, 2 H), and 8.03 (*dd*, $J = 8.0$, 1.5 Hz, 2 H)) and one typical *ABX* system (δ 6.94 (*d*, $J = 8.0$ Hz, 1 H), 6.53 (*d*, $J = 3.0$ Hz, 1 H), and 6.42 (*dd*, $J = 8.0$, 3.0 Hz, 1 H)). In addition, *cis*-alkene protons at δ 5.59 (*dt*, $J = 10.0$, 2.0 Hz, 1 H) and 6.03 (*dt*, $J = 10.0$, 4.0 Hz, 1 H), and protons at δ 2.72–2.75, 2.39–2.43, 2.50–2.55, and 2.32–2.36 (each *m*, each 1 H) were attributed to a (1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl moiety. A detailed comparison of the ^{13}C-NMR data of **2** (*Table 3*) with

Table 1. *^{1}H-NMR Data* (500 MHz, CD_3OD) *of* **1**, **10**, **3**, *and* **4**. *J* in Hz. Arbitrary atom numbering.

	1	**10**	**3**	**4**
H–C(3)	6.98 (*d*, *J* = 8.5)	7.06 (*d*, *J* = 8.5)	7.09 (*d*, *J* = 8.5)	7.08 (*d*, *J* = 8.5)
H–C(4)	6.58 (*dd*, *J* = 8.5, 3.0)	6.66 (*dd*, *J* = 8.5, 3.0)	6.69 (*dd*, *J* = 8.5, 3.0)	6.67 (*dd*, *J* = 8.5, 3.0)
H–C(6)	6.77 (*d*, *J* = 3.0)	6.60 (*d*, *J* = 3.0)	6.70 (*d*, *J* = 3.0)	6.70 (*d*, *J* = 3.0)
CH_2(7)	4.24, 4.50 (2*d*, *J* = 14.0)	4.85, 4.96 (2*d*, *J* = 12.5)	5.27 (*d*, *J* = 12.5)	5.26 (*d*, *J* = 12.5)
H–C(1′)	5.05 (*d*, *J* = 8.0)	5.05 (*d*, *J* = 8.0)	4.85 (*d*, *J* = 8.0)	4.82 (*d*, *J* = 8.0)
H–C(2′)	5.22 (*t*, *J* = 8.0)	5.21 (*t*, *J* = 8.0)	3.66–3.69 (*m*)	3.53–3.55 (*m*)
H–C(3′)	3.73–3.76 (*m*)	3.73–3.76 (*m*)	5.28 (*t*, *J* = 8.0)	3.53–3.55 (*m*)
H–C(4′)	3.50–3.54 (*m*)	3.51–3.55 (*m*)	3.66–3.70 (*m*)	5.05 (*t*, *J* = 8.5)
H–C(5′)	3.47–3.50 (*m*)	3.47–3.50 (*m*)	3.47–3.50 (*m*)	3.67–3.71 (*m*)
CH_2(6′)	3.93 (*dd*, *J* = 2.0, 6.0), 3.74 (*dd*, *J* = 6.0, 11.5)	3.94 (*dd*, *J* = 2.0, 6.0), 3.75 (*dd*, *J* = 6.0, 11.5)	3.85–3.87, 3.70–3.74 (2*m*)	3.53–3.55, 3.76–3.79 (2*m*)
H–C(2″,6″)	8.08 (*d*, *J* = 8.0)	8.06–8.09 (*m*)	8.05 (br. *d*, *J* = 8.5)	8.02 (br. *d*, *J* = 8.5)
H–C(3″,5″)	7.48 (*t*, *J* = 8.0)	7.46–7.49 (*m*)	7.46 (br. *t*, *J* = 8.5)	7.45 (br. *t*, *J* = 8.5)
H–C(4″)	7.60 (*t*, *J* = 8.0)	7.59–7.61 (*m*)	7.56 (br. *t*, *J* = 8.5)	7.60 (br. *t*, *J* = 8.5)
H–C(2‴)	–	5.68 (*dt*, *J* = 10.0, 16.0)	5.72 (*dt*, *J* = 10.0, 2.0)	5.73 (*dt*, *J* = 10.0, 2.0)
H–C(3‴)	–	6.10–6.14 (*m*)	6.10–6.14 (*m*)	6.11–6.14 (*m*)
CH_2(4‴)	–	2.61–2.64, 2.45–2.52 (2*m*)	2.62–2.64, 2.46–2.49 (2*m*)	2.62–2.64, 2.48–2.51 (2*m*)
CH_2(5‴)	–	2.80–2.87, 2.49–2.53 (2*m*)	2.82–2.86, 2.50–2.54 (2*m*)	2.83–2.88, 2.51–2.55 (2*m*)

those of 4-hydroxytremulacin (=4-hydroxy-2-{{[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl}phenyl β-D-glucopyranoside 2-benzoate; **10**) [3][6] suggested a strong similarity of **2** and **10**, except for an additional benzoyl fragment in **2**. The downfield shift of C(6′) from δ 62.5 to 65.2 in **2** established that this second benzoyl moiety was attached to OC(6′). This was further confirmed by the HMBC experiment, showing a long-range correlation δ(H) 4.72 and 4.44 (CH_2(6′))/δ(C) 167.7 (C(7‴)) (*Fig. 1*).

Compounds **3** and **4** were obtained as white amorphous powders. The molecular formulas were established as $C_{27}H_{28}O_{12}$ by the pseudo-molecular-ion peaks at *m*/*z* 567.1475 ($[M + Na]^+$; **3**) and 567.1476 ($[M + Na]^+$; **4**) in the HR-ESI-MS. Their ^{1}H- (*Table 1*) and ^{13}C-NMR (*Table 2*) data were similar to those of **10**, except for the chemical shifts of the glucose moiety [3][6], which indicated that the benzoyl moiety was attached to different positions. The structure of compound **3** was established as 4-hydroxy-2-{{[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl}phenyl β-D-glucopyranoside 3-benzoate and named itoside C, and that of **4** was determined as the corresponding 4-benzoate and named itoside D.

In compound **3**, the downfield shift of C(3′) to δ 79.8, and the upfield shifts of C(2′) and C(4′) to δ 73.4 and 69.6 compared with 4-hydroxytremulacin (**10**) [3][6] showed the attachment of the benzoyl moiety at OC(3′). In compound **4**, the downfield shift of C(4′) to δ 72.9, and the upfield shifts of C(3′) and (5′) to δ 75.8 and 76.1 indicated that the benzoyl fragment was attached to OC(4′). HMBC Experiments showed the long-range correlations δ(H) 5.28 (H–C(3′))/δ(C) 167.9 (C(7″)) in **3** and δ(H) 5.05 (H–C(4′))/δ(C) 167.4 (C(7″)) in **4**.

Compound **5** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{27}H_{26}O_{11}$ by the pseudo-molecular-ion peak at *m*/*z* 527.1542

Table 2. *^{13}C-NMR Data* (125 MHz) *of* **1**, **10**, *and* **3**–**8**. δ in ppm. Arbitrary atom numbering.

	1[a])	**10**[a])	**3**[a])	**4**[a])	**5**[b])	**6**[a])	**7**[b])	**7**[a])	**8**[a])
C(1)	133.3	127.6	128.0	127.9	126.8	128.3	127.6	128.3	129.0
C(2)	148.9	149.6	149.9	150.0	148.7	150.0	150.2	150.0	149.7
C(3)	118.4	119.5	119.7	119.6	118.3	119.0	119.5	119.6	120.3
C(4)	115.2	116.9	117.0	117.0	116.8	116.9	116.9	116.9	116.7
C(5)	154.2	154.2	154.1	154.1	154.4	154.2	154.6	154.2	154.3
C(6)	115.2	116.4	116.8	116.8	116.5	116.5	116.6	116.5	116.3
C(7)	59.9	63.9	64.3	64.4	62.2	63.4	63.0	63.5	63.4
C(1′)	102.2	102.2	104.2	104.2	101.5	104.4	104.7	104.3	104.8
C(2′)	75.7	75.7	73.4	75.2	75.7	73.4	75.1	75.2	75.0
C(3′)	76.0	76.1	79.6	75.8	76.0	79.7	76.0	76.0	78.0
C(4′)	71.7	71.6	69.6	72.9	71.5	69.6	73.0	72.9	72.0
C(5′)	78.3	78.4	78.0	76.1	79.1	78.0	76.5	76.2	75.5
C(6′)	62.5	62.5	62.3	62.3	62.4	63.4	62.0	62.3	65.3
C(1″)	131.4	131.3	129.3	129.4	130.4	131.3	130.6	131.3	131.3
C(2″,6″)	130.8	130.8	130.8	130.8	130.1	130.8	130.1	130.3	130.6
C(3″,5″)	129.6	129.6	129.5	129.6	128.7	129.5	128.8	129.6	129.6
C(4″)	134.4	134.4	134.2	134.4	133.2	134.2	133.3	134.5	134.3
C(7″)	167.3	167.2	167.9	167.4	166.2	167.9	166.2	167.4	167.8
C(1‴)		79.2	79.4	79.3	113.0	113.8	113.2	113.8	113.7
C(2‴)		129.2	129.3	129.4	161.9	162.8	162.0	162.8	162.8
C(3‴)		133.3	133.4	133.3	117.7	118.4	117.8	118.4	118.3
C(4‴)		27.2	27.3	27.3	135.9	136.9	136.0	136.9	136.8
C(5‴)		36.8	36.9	36.9	120.2	120.4	119.6	120.4	120.2
C(6‴)		207.3	207.3	207.3	130.8	131.8	130.9	131.8	131.2
C(7‴)		171.2	171.5	171.5	169.9	171.2	170.2	171.2	171.1

[a]) Measured in CD_3OD. [b]) Measured in (D_5)pyridine.

([M + H]$^+$). The IR spectrum exhibited the absorption bands of OH (3410 cm^{-1}) and C=O groups (1710 and 1673 cm^{-1}). A benzoyl moiety, an esterified gentisyl alcohol (=2-(hydroxymethyl)benzene-1,4-diol) moiety and a 2-hydroxybenzoyl moiety were identified from the ^{13}C-NMR data (*Table 2*) and 2D-NMR experiments. The presence of a D-glucose moiety was established by the NMR data and confirmed by GC analysis after acid hydrolysis. The structure of **5** was established as 4-hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-D-glucopyranoside 2-benzoate and named itoside E.

The ^{1}H-NMR spectrum of **5** (*Table 4*) showed one monosubstituted benzene ring (δ 8.25 (*dd*, J = 7.5, 2.5 Hz, 2 H), 7.28 (*dd*, J = 7.5, 2.5 Hz, 2 H), and 7.39 (*dt*, J = 7.5, 2.5 Hz, 1 H)), one *ABX* system (δ 7.58 (*d*, J = 9.0 Hz, 1 H), 7.05 (*dd*, J = 9.0, 2.5 Hz, 1 H), and 7.25 (*d*, J = 2.5 Hz, 1 H)) and one *AA′BB′* system (δ 7.02 (br. *d*, J = 8.0 Hz, 1 H), 7.35 (br. *t*, J = 8.0 Hz, 1 H), 6.67 (br. *t*, J = 8.0 Hz, 1 H), and 7.66 (br. *d*, J = 8.0 Hz, 1 H)). Comparing the NMR data of **5** with those of **3**, there was an obvious downfield shift of H–C(2′) to δ(H) 6.10 and of C(2′) to δ(C) 75.7. Upfield shifts of C(1′) to δ(C) 101.5 and C(3′) to δ(C) 76.0 were also observed, which suggested that the glucose unit of **5** was esterified at C(2′). The long-range correlation δ(H) 6.10 (H–C(2′))/δ(C) 166.2 (C(7″)) (*Fig. 2*) in the HMBC experiment indicated that the benzoyl moiety was attached to C(2′). Long-range correlations δ(H) 5.68 (H–C(1′))/δ(C) 148.7 (C(2)), δ(H) 5.55 (CH$_2$(7))/δ(C) 169.9 (C(7‴)) (*Fig. 2*) were observed, indicating that C(1′) was

Table 3. *NMR Data* (500 (^{1}H) and 125 MHz (^{13}C), CD_3OD) *of* **2**. δ in ppm, J in Hz. Arbitrary atom numbering.

	δ(C)	δ(H)		δ(C)	δ(H)
C(1)	127.9	–	H–C(2″,6″)	130.8	8.03 (*dd*, *J* = 1.5, 8.0)
C(2)	149.3	–	H–C(3″,5″)	129.7	7.43–7.50 (*m*)
H–C(3)	119.5	6.94 (*d*, *J* = 8.0)	H–C(4″)	134.4	7.52–7.59 (*m*)
H–C(4)	116.9	6.42 (*dd*, *J* = 8.0, 3.0)	C(7″)=O	167.2	–
C(5)	154.3	–	C(1‴)	131.1	–
H–C(6)	116.5	6.53 (*d*, *J* = 3.0)	H–C(2‴,6‴)	130.6	7.98 (*dd*, *J* = 1.5, 8.0)
CH_2(7)	63.9	4.85 (*d*, *J* = 12.5),	H–C(3‴,5‴)	129.6	7.43–7.50 (*m*)
		4.96 (*d*, *J* = 12.5)	H–C(4‴)	134.4	7.52–7.59 (*m*)
H–C(1′)	102.2	5.05 (*d*, *J* = 8.0)	C(7‴)=O	167.7	–
H–C(2′)	75.6[a])	5.21 (*t*, *J* = 8.0)	C(1‴)	79.2	–
H–C(3′)	76.0	3.78–3.80 (*m*)	H–C(2‴)	129.2	5.59 (*dt*, *J* = 10.0, 2.0)
H–C(4′)	72.2	3.56 (*t*, *J* = 8.0)	H–C(3‴)	133.3	6.03 (*dt*, *J* = 10.0, 4.0)
H–C(5′)	75.7[a])	3.78–3.80 (*m*)	CH_2(4‴)	27.2	2.50–2.55, 2.32–2.36 (2*m*)
CH_2(6′)	65.2	4.72 (*dd*, *J* = 7.0, 2.0),	CH_2(5‴)	36.8	2.39–2.43, 2.72–2.75 (2*m*)
		4.44 (*dd*, *J* = 7.0, 11.5)	C(6‴)=O	207.3	–
C(1″)	131.3	–	C(7‴)=O	171.2	–

[a]) Assignment maybe exchanged.

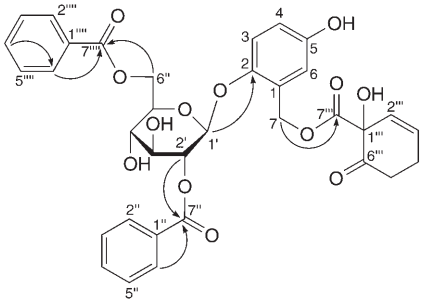

Fig. 1. *Key HMBC correlations of* **2**

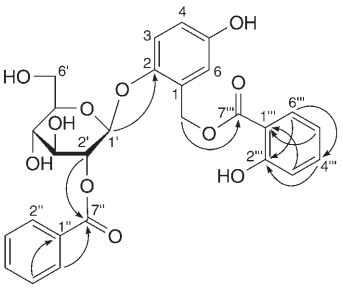

Fig. 2. *Key HMBC correlations of* **5**

connected at C(2), and C(7) of the gentisyl alcohol moiety was esterified by 2-hydroxybenzoic acid.

Table 4. *^{1}H-NMR Data* (500 MHz) *of* **5**–**8**. δ in ppm, J in Hz. Arbitrary atom numbering.

	5[a])	**6**[b])	**7**[a])	**7**[b])	**8**[b])
H–C(3)	7.58 (*d*, *J* = 9.0)	7.16 (*d*, *J* = 8.5)	7.68 (*d*, *J* = 8.5)	7.16 (*d*, *J* = 8.0)	7.06 (*d*, *J* = 8.5)
H–C(4)	7.05 (*dd*, *J* = 9.0, 2.5)	6.75 (*dd*, *J* = 8.5, 3.0)	7.10 (*dd*, *J* = 8.5, 3.5)	6.74 (*dd*, *J* = 8.0, 3.0)	6.57 (*dd*, *J* = 8.5, 3.0)
H–C(6)	7.25 (*d*, *J* = 2.5)	6.87 (*d*, *J* = 3.0)	7.38 (*d*, *J* = 2.5)	6.88 (*d*, *J* = 3.0)	6.82 (*d*, *J* = 3.0)
CH_2(7)	5.55 (*s*)	5.53 (*dd*, *J* = 12.5, 12.5)	5.84 (*dd*, *J* = 12.5, 12.5)	5.47 (*dd*, *J* = 12.5, 12.5)	5.45 (*d*, *J* = 13.0, 13.0)
H–C(1′)	5.68 (*d*, *J* = 8.0)	4.95 (*d*, *J* = 8.0)	5.52 (*d*, *J* = 8.0)	4.90 (*d*, *J* = 8.0)	4.77 (*d*, *J* = 7.5)
H–C(2′)	6.10 (*t*, *J* = 8.0)	3.77 (*t*, *J* = 8.0)	4.37 (*t*, *J* = 8.0)	3.65–3.68 (*m*)	3.46–3.49 (*m*)
H–C(3′)	4.51–4.55 (*m*)	5.28 (*t*, *J* = 8.0)	4.48–4.52 (*m*)	3.80–3.84 (*m*)	3.45–3.47 (*m*)
H–C(4′)	4.37–4.40 (*m*)	3.56–3.59 (*m*)	5.98 (*t*, *J* = 9.0)	5.11 (*t*, *J* = 9.5)	3.40–3.45 (*m*)
H–C(5′)	4.16–4.19 (*m*)	3.51–3.53 (*m*)	4.21–4.24 (*m*)	3.66–3.70 (*m*)	3.65–3.69 (*m*)
CH_2(6′)	4.38–4.40, 4.55–4.59 (2*m*)	3.72–3.76, 3.87–3.91 (2*m*)	4.15–4.18, 4.23–4.26 (2*m*)	3.52–3.56, 3.75–3.79 (2*m*)	4.67 (*dd*, *J* = 2.5, 12.0), 4.37 (*dd*, *J* = 7.5, 12.0)
H–C(2″,6″)	8.25 (*dd*, *J* = 7.5, 2.5)	8.09 (*dd*, *J* = 8.0, 2.5)	8.17 (br. *d*, *J* = 8.0)	8.05 (br. *d*, *J* = 8. 5)	7.97 (*dd*, *J* = 8.0, 2.5)
H–C(3″,5″)	7.28 (*dd*, *J* = 7.5, 2.5)	7.45–7.49 (*m*)	7.34 (br. *t*, *J* = 8.0)	7.46 (*dd*, *J* = 8.5)	7.46 (br. *t*, *J* = 8.0)
H–C(4″)	7.39 (*dt*, *J* = 7.5, 2.5)	7.60 (*dt*, *J* = 8.0, 2.5)	7.45 (br. *t*, *J* = 8.0)	7.59–7.63 (*m*)	7.58 (*dt*, *J* = 8.0, 2.5)
H–C(3‴)	7.02 (br. *d*, *J* = 8.0)	6.93 (*dd*, *J* = 8.0, 1.0)	7.05 (br. *d*, *J* = 8.0)	6.94 (br. *d*, *J* = 8.0)	6.90 (*dd*, *J* = 8.0, 1.5)
H–C(4‴)	7.35 (br. *t*, *J* = 8.0)	7.46–7.49 (*m*)	7.39 (br. *t*, *J* = 8.0)	7.47–7.49 (*m*)	7.42–7.46 (*m*)
H–C(5‴)	6.67 (br. *t*, *J* = 8.0)	6.88–6.90 (*m*)	6.76 (br. *t*, *J* = 8.0)	6.87–6.89 (*m*)	6.85 (*d t*, *J* = 8.0, 1.5)
H–C(6‴)	7.66 (br. *d*, *J* = 8.0)	7.91 (*dd*, *J* = 8.0, 1.0)	7.85 (br. *d*, *J* = 8.0)	7.92 (br. *d*, *J* = 8.0)	7.83 (*dd*, *J* = 8.0, 1.5)

[a]) Measured in (D_5)pyridine. [b]) Measured in CD_3OD.

Compounds **6**–**8** were obtained as amorphous powders. Their molecular formulas were determined to be $C_{27}H_{26}O_{11}$ by HR-ESI-MS, the same as that of **5**. Their ^{1}H- (*Table 4*) and ^{13}C-NMR (*Table 2*) showed high similarity with **5**, except for the signals of the glucose residue. Therefore, it was deduced that **5**, **6**, **7**, and **8** varied only in the benzoyl position at the glucose unit. Compound **6** was determined to be 4-hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-D-glucopyranoside 3-benzoate and named itoside F, compound **7** was the corresponding 4-benzoate and named itoside G, and compound **8** was the corresponding 6-benzoate and named itoside H.

The downfield shift of the glucose C(3′) of **6** to δ 79.7 and the upfield shift of C(2′) and C(4′) to δ 73.4 and 69.6 as compared to **5**, together with the long-range correlation δ(H) 5.28 (H–C(3′))/δ(C) 167.9 (C(7″)), indicated the attachment position of the benzoyl moiety at OC(3′). Similarly, a downfield shift of C(4′) of **7** to δ 72.9 and an upfield shift of C(3′) and C(5′) to δ 76.0 and 76.2, as well as a downfield shift of C(6′) of **8** to δ 65.3 and an upfield shift of C(5′) to δ 75.5 were observed. The HMBC cross-peaks δ(H) 5.11 (H–C(4′))/δ(C) 167.4 (C(7″)) of **7** and δ(H) 4.67 and 4.37 (CH_2(6′))/δ(C) 167.8 (C(7″)) of **8** confirmed that the benzoyl moiety was connected at OC(4′) in compound **7** and at OC(6′) in **8**.

Compound **9** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{18}H_{26}O_{11}$ by the pseudo-molecular-ion peak at *m*/*z* 457.1107 ($[M + K]^+$) in the HR-ESI-MS. It showed UV absorptions at 194, 273, and 202 nm and IR bands at 3387, 1597, and 1500 cm^{-1}. The identification of the sugar units D-glucose and L-rhamnose was confirmed by TLC and GC analyses after acid hydrolysis. Compound **9** was established as 2-hydroxyphenyl *O*-6-deoxy-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, and named itoside I.

The ^{1}H-NMR spectrum of **9** (*Table 5*) exhibited signals for a 1,2-disubstituted benzene ring (δ 6.80 (*dd*, *J* = 8.0, 1.5 Hz), 6.73 (*dt*, *J* = 8.0, 1.5 Hz), 6.87 (*dt*, *J* = 8.0, 1.5 Hz), and 7.12 (*dd*, *J* = 8.0, 1.5 Hz)), a β-D-glucopyranose moiety (δ(H) 4.69 (*d*, *J* = 7.0 Hz (H–C(1′)) and an α-L-rhamnopyranose moiety (δ(H) 4.72 (*d*, *J* = 2.0 Hz, H–C(1″)). The ^{13}C-NMR spectrum exhibited the signals of six aromatic C-atoms, besides those of two quaternary C-atoms at δ 147.2 (C(1)) and 149.9 (C(2)). These data were similar to those of pyrocatechol β-D-glucopyranoside [7], indicating a 1,2-dioxygenated benzene (benzene-1,2-diol) moiety. In the HMBC plot, the key correlations δ(H) 4.69 (H–C(1′))/δ(C) 147.2 (C(1)) and δ(H) 4.72 (H–C(1″))/δ(C)) 67.9 (C(6′)) were observed (*Fig. 3*), which confirmed the glycosidic linkages.

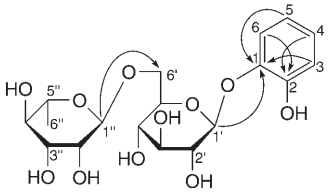

Fig. 3. *Key HMBC correlations of* **9**

Phenolic glycosides are major constituents in plants of the family Flaourtiaceae [8]. Gentisyl alcohol glycosides have been in particular found in the genera *Poliothyrsis* and *Homalium*. However, compounds such as **5**–**8**, in which the gentisyl alcohol moiety is esterified by a 2-hydroxybenzoic acid residue, were now found for the first time in this plant family.

Table 5. *NMR Data* (500 (^{1}H) and 125 MHz (^{13}C), CD_3OD) *for* **9**. δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC
C(1)	147.2		
C(2)	149.9		
H–C(3)	117.7	6.80 (*dd*, *J* = 8.0, 1.5)	C(1), C(2), C(4), C(5)
H–C(4)	124.9	6.87 (*dt*, *J* = 8.0, 1.5)	C(2), C(3), C(5), C(6)
H–C(5)	120.1	6.73 (*dt*, *J* = 8.0, 1.5)	C(1), C(3), C(4), C(6)
H–C(6)	118.9	7.12 (*dd*, *J* = 8.0, 1.5)	C(1), C(2), C(4), C(5)
H–C(1′)	104.4	4.69 (*d*, *J* = 7.0)	C(1), C(3′), C(5′)
H–C(2′)	74.9	3.48 (*t*, *J* = 7.0)	C(1′)
H–C(3′)	77.7	3.44 (*t*, *J* = 7.0)	C(2′), C(4′)
H–C(4′)	71.6	3.38 (*t*, *J* = 7.0)	C(3′), C(5′)
H–C(5′)	77.1	3.52 (*dt*, *J* = 7.0, 2.0)	C(1′)
CH_2(6′)	67.9	4.02 (*dd*, *J* = 11.0, 2.0), 3.61 (*dd*, *J* = 11.0, 6.5)	C(4′), C(1″)
H–C(1″)	102.2	4.72 (*d*, *J* = 2.0)	C(6′), C(3″), C(5″)
H–C(2″)	72.2	3.85 (*dd*, *J* = 1.5, 3.5)	C(1″)
H–C(3″)	72.4	3.69 (*dd*, *J* = 9.0, 3.5)	C(2″), C(4″)
H–C(4″)	74.0	3.38 (*t*, *J* = 9.0)	C(3″), C(5″)
H–C(5″)	69.8	3.64–3.67 (*m*)	C(1″)
Me(6″)	18.0	1.23 (*d*, *J* = 7.0)	C(4″)

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh) and silica gel *H* (400–600 mesh) (*Qingdao Mar. Chem. Ind. Co. Ltd.*), *Sephadex-LH-20* gel (*Pharmacia*), octadecyl silical gel (= *ODS*; 25–40 μm; *Merck*); a N_2 pressure of 0.12 MPa was applied. GC: *Agilent 6890N*; *HP-5* capillary column (28 m × 0.32 mm, i.d.); detection by FID; detector temp. 260°; column temp. 180°; carrier gas N_2; flow rate 40 ml/min). Melting points: *XT-4A* digital micro-melting point apparatus. Optical rotations: *Perkin-Elmer 243B* digital polarimeter. UV Spectra: *Shimadzu UV-2450* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nexus-470-FTIR* (*Nicolet*) spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra, HMQC, HMBC, and ^{1}H,^{1}H-COSY: *Bruker DRX-400* spectrometer; δ in ppm, *J* in Hz. MS: *QSTAR* mass spectrometer for ESI and *Bruker APEX-IV-FTMS* spectrometer for HR-ESI; in *m*/*z*.

Plant Material. The barks and twigs of *Itoa orientalis* HEMSL. were collected in Guangxi Province of China, and authenticated by Mr. *Maojing Yang*, from the Chinese Academy of Forestry. A voucher specimen (IO20041205) is kept in the herbarium of the Peking University Modern Research Centre for Traditional Chinese Medicine.

Extraction and Isolation. The dried barks (18 kg) and twigs (22 kg) of *Itoa orientalis* were extracted twice with boiling 80% EtOH (2 × 400 l), each time for 2 h. After evaporation of the solvent at 60°, the residue was suspended in H_2O (1.8 l) and extracted successively with $CHCl_3$ (2 × 5 l), AcOEt (2 × 5 l), and BuOH (2 × 5 l). The BuOH extract (520 g) was subjected to CC (silica gel (14 × 130 cm), $CHCl_3$/MeOH 20:1 → 0:1): *Fractions 1–7. Fr. 3* was resubjected to CC (silica gel, AcOEt/MeOH 40:1): *Fr. 3A–3E. Fr. 3D* (12 g) was subjected CC (*Sephadex LH-20*, MeOH): **5** (15 mg) and tremulacin [3][6] (230 mg). *Fr. 2* was resubjected to CC (silica gel, $CHCl_3$/MeOH 20:1): *Fr. 2A–2D. Fr. 2B* was further purified by CC (*Sephadex LH-20*): *homaloside D* (**13**; 2.5 g). *Fr. 2C* (7 g) was subjected to CC (*Sephadex LH-20*, MeOH): *4-hydroxytremulacin* (**10**; 1500 mg). *Fr. 4* was resubjected to CC (silica gel, AcOEt/

MeOH 40:1): *Fr. 4.I–4.VIII*. *Fr. 4.IV* was subjected to CC (silica gel, AcOEt/MeOH 30:1; then *Sephadex LH-20*, MeOH): **1** (1020 mg), *poliothyrsin* (**12**; 2.1 g) and *poliothyrsoside* (**11**; 6.5 g). *Fr. 6* was subjected to CC (silica gel, AcOEt/MeOH/H_2O 10:1:0.1 → 0:1:0): *Fr. 6.I–6.XII*. Purification of *Fr. 6.VI* by CC (*Sephadex LH-20*; then *ODS*, MeOH/H_2O 4:6) gave **9** (120 mg). *Fr. 6.V* was subjected to CC (*Sephadex LH-20*, MeOH/H_2O 8:2): to give *pyrocatechol β-*D*-glucopyranoside* (2 g). *Fr. 1* (3 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 40:1): *Fr. 1.1–1.6*. *Fr. 1.2* was subjected to CC (*Sephadex LH-20*, MeOH): **2** (151 mg). *Fr. 1.4* was subjected to CC (silica gel, petroleum ether/Me_2CO 1:1): *Fr. 1.4.1–1.4.2*. *Fr. 1.4.1* was subjected to CC (*Sephadex LH-20*, MeOH; then *ODS*, MeOH/H_2O 55:45): **6** (33 mg) and **8** (30 mg). *Fr. 1.4.2* was subjected to CC (silica gel *H*, $CHCl_3$/MeOH 15:1): **6** (13 mg) and **7** (22 mg). *Fr. 1.5* (1.5 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 12:1; then *Sephadex LH-20*, MeOH): **3** (22 mg) and **4** (30 mg).

Itoside A (=*4-Hydroxy-2-(hydroxymethyl)phenyl β-*D*-Glucopyranoside 2-Benzoate*; **1**): White amorphous powder. $[\alpha]_D^{25} = -10.5$ ($c = 0.20$, MeOH). UV (MeOH): 203 (4.68), 227 (4.60), 282 (3.86). IR (KBr): 3434, 2969, 1716, 1501, 1453, 1284, 1085, 1034, 7.98. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. ESI-MS: 429 ($[M+Na]^+$). HR-ESI-MS: 429.1156 ($[M+Na]^+$; calc. 429.1151).

Itoside B (=*4-Hydroxy-2-{[[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl]phenyl β-*D*-Glucopyranoside 2,6-Dibenzoate*; **2**): Colorless syrup. $[\alpha]_D^{25} = -97.5$ ($c = 0.23$, MeOH). UV (MeOH): 203 (4.70), 229 (4.71), 282 (3.96). IR (KBr): 3425, 2925, 1721, 1602, 1451, 1273, 1073, 714. ^{1}H- and ^{13}C-NMR: *Table 3*. ESI-MS: 617 ($[M+Na]^+$). HR-ESI-MS: 617.1714 ($[M+Na]^+$; calc. 617.1735).

Itoside C (=*4-Hydroxy-2-{[[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl]phenyl β-*D*-Glucopyranoside 3-Benzoate*; **3**): Amorphous powder. $[\alpha]_D^{25} = -94.2$ ($c = 0.19$, MeOH). UV (MeOH): 203 (4.77), 228 (4.73), 285 (3.94). IR (KBr): 3427, 2927, 1720, 1602, 1500, 1451, 1274, 1073, 714. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. HR-ESI-MS: 567.1475 ($[M+Na]^+$; calc. 567.1473).

Itoside D (=*4-Hydroxy-2-{[[(1-hydroxy-6-oxocyclohex-2-en-1-yl)carbonyl]oxy}methyl]phenyl β-*D*-Glucopyranoside 4-Benzoate*; **4**): Amorphous powder. $[\alpha]_D^{25} = -109.1$ ($c = 0.22$, MeOH). UV (MeOH): 203 (4.77), 228 (4.73), 285 (3.94). IR (KBr): 3425, 2926, 1721, 1602, 1500, 1452, 1271, 1073, 715. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. HR-ESI-MS: 567.1476 ($[M+Na]^+$; calc. 567.1473).

Itoside E (=*4-Hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-*D*-Glucopyranoside 2-Benzoate*; **5**): White amorphous powder. $[\alpha]_D^{25} = -20.0$ ($c = 0.10$, MeOH). UV (MeOH): 204 (4.93), 228 (4.66), 301 (4.22). IR (KBr): 3410, 2932, 1710, 1673, 1612, 1491, 1297, 1077, 711. ^{1}H-NMR: *Table 4*. ^{13}C-NMR: *Table 2*. HR-ESI-MS: 527.1542 ($[M+H]^+$; $C_{27}H_{27}O_{11}^+$; calc. 527.1548).

Itoside F (=*4-Hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-*D*-Glucopyranoside 3-Benzoate*; **6**): White amorphous powder. $[\alpha]_D^{25} = -4.0$ ($c = 0.10$, MeOH). UV (MeOH): 194 (4.21), 203 (4.77), 229 (4.48), 296 (3.80). IR (KBr): 3412, 2931, 1711, 1674, 1611, 1492, 1298, 1077, 711. ^{1}H-NMR: *Table 4*. ^{13}C-NMR: *Table 2*. ESI-MS: 549 ($[M+Na]^+$). HR-ESI-MS: 527.1544 ($[M+H]^+$, calc. 527.1548).

Itoside G (=*4-Hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-*D*-Glucopyranoside 4-Benzoate*; **7**): White amorphous powder. $[\alpha]_D^{25} = -26.0$ ($c = 0.13$, MeOH). UV (MeOH): 194 (4.42), 204 (4.97), 229 (4.69), 296 (4.06). IR (KBr): 3410, 2930, 1709, 1673, 1610, 1493, 1297, 1078, 711. ^{1}H-NMR: *Table 4*. ^{13}C-NMR: *Table 2*. ESI-MS: 549 ($[M+Na]^+$). HR-ESI-MS: 527.1545 ($[M+H]^+$, $C_{27}H_{27}O_{11}^+$; calc. 527.1548).

Itoside H (=*4-Hydroxy-2-{[(2-hydroxybenzoyl)oxy]methyl}phenyl β-*D*-Glucopyranoside 6-Benzoate*; **8**): White amorphous powder. $[\alpha]_D^{25} = -17.0$ ($c = 0.20$, MeOH). UV (MeOH): 205 (4.88), 228 (4.61), 297 (4.00). IR (KBr): 3414, 2928, 1710, 1673, 1610, 1493, 1298, 1078, 711. ^{1}H-NMR: *Table 4*. ^{13}C-NMR: *Table 2*. ESI-MS: 549 ($[M+Na]^+$). HR-ESI-MS: 527.1543 ($[M+H]^+$; calc. 527.1548).

Itoside I (=*2-Hydroxyphenyl* O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 6)-β-*D*-glucopyranoside*; **9**): White amorphous powder. $[\alpha]_D^{25} = -40.5$ ($c = 0.20$, MeOH). UV (MeOH): 194 (3.76), 202 (4.37), 273 (3.66). IR (KBr): 3387, 2925, 1597, 1500, 1268, 1069. ^{1}H- and ^{13}C-NMR: *Table 5*. ESI-MS: 441 ($[M+Na]^+$). HR-ESI-MS: 457.1107 ($[M+K]^+$; calc. 457.1104).

Acid Hydrolysis and Sugar Analyses. Each compound **1**, **5**, or **9** (5 mg) was heated in 10% HCl/dioxane 1:1 (5 ml) at 80° for 4 h. After evaporation of the dioxane, the soln. was extracted with AcOEt (3×3 ml). The aq. layer was neutralized with $NaHCO_3$ and concentrated. A small amount of the solid residue was dissolved in MeOH and analyzed by TLC ($CHCl_3$/MeOH/H_2O 8:5:1; detection by spraying

with 95% EtOH/H_2SO_4/anisaldehyde 9 : 0.5 : 0.5 (*v*/*v*), followed by heating at 120° for 10 min): glucose (R_f 0.30) from **1**, **5**, and **9**, and rhamnose (R_f 0.50) from **9**.

The TLC results were confirmed by GC analyses. The solid residue from the aq. layer was dissolved in anh. pyridine (100 µl), and 0.1M L-cysteine methyl ester hydrochloride (200 µl) was added and the mixture warmed at 60° for 1 h. Then hexamethyldisilazane (HMDS)/chlorotrimethylsilane/pyridine 2 : 1 : 10 (*Acros Organics*, Belgium) was added and the mixture warmed at 60° for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification: D-glucose derivative (t_R 12.45 min) and L-rhamnose derivative (t_R 5.32 min).

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