Itosides J−N from *Itoa orientalis* and Structure−Anti-COX-2 Activity Relationship of Phenolic Glycosides

Xing Yun Chai, Yue Lin Song, Zheng Ren Xu, Hai Ming Shi, Chang Cai Bai, Dan Bi, Jing Wen, Fei Fei Li, and Peng Fei Tu*

Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, No. 38, Xueyuan Road, Haidian District, Beijing, 100083, People's Republic of China

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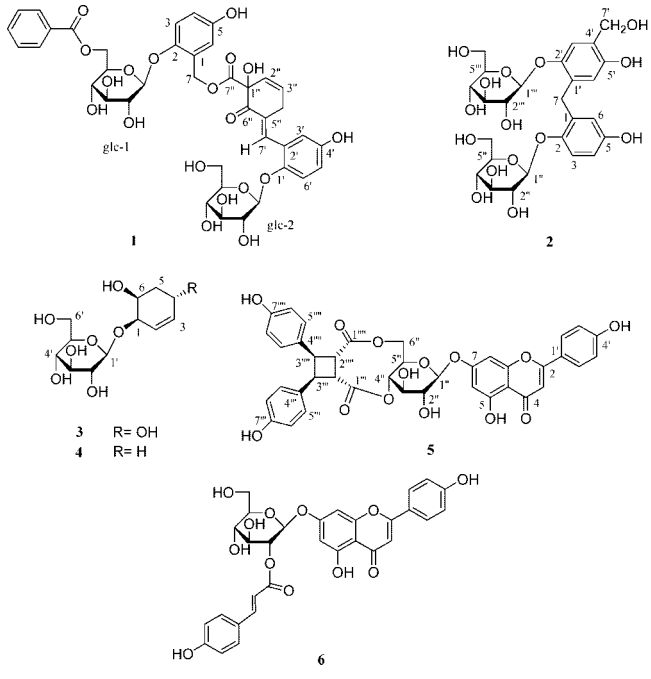
Two new phenolic glycosides, itosides J (**1**) and K (**2**), two new cylcohexenoyl glycosides, itosides L (**3**) and M (**4**), a new flavone glycoside, itoside N (**5**), and echitin (**6**) were isolated from the extract of the bark, twigs, and leaves of *Itoa orientalis*, together with 22 known compounds. The structures were elucidated by means of UV, IR, MS, and NMR techniques, and the relative configuration of compound **3** was confirmed by X-ray crystallography. NMR data for **6** are reported for the first time. Compounds **1**, **3**, **5**, and phenolic glycosides **7**−**22** were also assayed for anti-inflammatory activity against COX-2. Compounds **8**, **10**, **12**−**14**, **16**, **19**, **24**, **26**, and **27** showed significant inhibitory effects, with inhibitory rates of 49.7−85.3% at 10 μM.

As part of our comprehensive investigation of bioactive compounds from Flacourtiaceae plants found in China, the constituents of *Itoa orientalis* were investigated. We previously reported a series of phenolic glycosides isolated from *I. orientalis* Hemsl. Flacourtiaceae.[1] Further investigation of chemical constituents of this plant led to the isolation and structural elucidation of five new compounds (**1**−**5**), echitin (**6**),[2] and 22 other known compounds. The structures of the new compounds were determined by means of IR, UV, MS, and 1D and 2D NMR spectra, and the structure of **3** was confirmed by X-ray crystallography. The NMR data of **6** are reported here for the first time.

Compounds **1**, **3**, **5**, and **22**−**28** and previously reported itosides A−I (**7**−**15**, respectively), 4-hydroxytremulacin (**16**), poliothyrsoside (**17**), poliothyrsin (**18**), homaloside D (**19**), tremulacin (**20**), and pyrocatechol-*O*-β-D-glucopyranoside (**21**)[1] were tested for anti-inflammatory activity against COX-2.



Results and Discussion

An 80% ethanol extract of the bark, twigs, and leaves of *I. orientalis* was suspended in H_2O and extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH. The aqueous layer was concentrated under reduced pressure, and the residue was repeatedly subjected to column chromatography on silica gel, Sephadex LH-20, and octadecylsilyl silica gel (ODS) to yield compounds **1**−**5** and 23 known compounds. The NMR data of the new compounds are presented in Tables 1−4, and the bioactivity results are summarized in Table 5.

Compound **1** was obtained as a yellow-green crystalline powder. Its molecular formula was determined to be $C_{40}H_{42}O_{19}$ by HRESIMS. The IR spectrum displayed absorption bands indicating OH (3404 cm^{-1}) and carbonyl (1704, 1708 cm^{-1}) groups. GC analysis showed the presence of D-glucose after acid hydrolysis of **1**. In the ^{1}H NMR spectrum of **1**, peaks characteristic of benzoyl, gentisyl, and glucose moieties were observed, suggesting a partial structure similar to that of poliothyrsoside (**17**).[3] HMBC correlations from H-1 of glc-1 to C-2, from H-6 (glc-1) to C-7 of benzoyl, and from H-5 (glc-1) to C-1 (glc-1) confirmed this conclusion. In addition, a 1,2,4-trisubstituted benzenoid system and another glucose unit, together with HMBC correlations from H-1 of glc-2 to C-1′, from H-6′ to C-1′, from C-2′ and C-4′, and from H-3′ to C-1′, C-2′, and C-5′, suggested the presence of a partial structure similar to salirepin (**23**).[4] An isolated −CH═CH−CH$_2$− unit was established by proton resonances at δ 5.78 (1H, dd, J = 10.0, 2.5 Hz), 6.11 (1H, m), and 3.30−3.50 (2H, m). The ^{13}C NMR spectrum showed the presence of four quaternary carbons, consistent with an oxygenated carbon (C-1″), a trisubstituted olefin, an additional carbonyl group (C-7″), and an α,β-unsaturated ketone (C-6″). HMBC correlations from H-4″ to C-2″, C-3″, C-6″, C-5″, and C-7′; from H-2″ to C-6″; from H-3″ to C-1″; and from H-7′ to C-3″ and C-6″ suggested a 1-hydroxy-6-oxocyclohex-2-enoic moiety as found in itosides B−D.[1] Comparing the ^{13}C NMR data with those of the cyclohexenyl unit in 4-hydroxytremulacin (**16**),[5] the only differences were an upfield shift of 9.7 ppm for C-6″ and a downfield shift of 3.3 ppm for C-4″, revealing the presence of a substituent on C-5″. Further HMBC correlations from H-7 to C-7″ and from H-7′ to C-1′ and C-3′ established the linkages as shown. A NOESY correlation was observed between H-4″ and H-3′, confirming an *E* configuration of the C-5″/C-7′ double bond. Thus, **1** is a novel phenolic glycoside, and it was named itoside J.

Compound **2** was obtained as a white, amorphous powder. HRESIMS suggested the molecular formula $C_{26}H_{34}O_{15}$. Its ^{1}H NMR spectrum (Table 2) indicated the presence of a 1,2,4-trisubstituted

* To whom correspondence should be addressed. Tel/fax: +86-10-8280 2750. E-mail: pengfeitu@vip.163.com.

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Table 1. NMR Data (500/125 MHz, in CD_3OD) for **1**, δ in ppm and J in Hz

no.	δ_C	δ_H	no.	δ_C	δ_H
genstisyl			benzoyl		
1	128.3		1	131.3	
2	149.8		2, 6	130.6	7.94 (2H, m)
3	120.2	6.98 (1H, d, 9.0)	3, 5	129.6	7.46 (2H, m)
4	117.0	6.50 (1H, dd, 9.0, 3.0)	4	134.3	7.59 (1H, m)
5	154.1		7	167.8	
6	117.1	6.67 (1H, d, 3.0)	glc-1		
7	64.4	5.19 (1H, d, 15.0)	1	104.3	4.67 (1H, d, 7.0)
		5.24 (1H, d, 15.0)	2	74.8	3.42−3.48 (1H, m)
phenyl	3	78.1	3.42−3.48 (1H, m)		
1′	151.5		4	71.9	3.42−3.48 (1H, m)
2′	127.4		5	75.4	3.65 (1H, m)
3′	117.4	6.72 (1H, d, 3.0)	6	65.2	4.66 (1H, dd, 12.0, 2.0)
4′	153.4				4.40 (1H, dd, 12.0, 5.5)
5′	118.6	6.76 (1H, dd, 8.5, 3.0)	glc-2		
6′	119.0	7.07 (1H, d, 8.5)	1	103.5	4.78 (1H, d, 7.5)
7′	135.9	8.00 (1H, brs)	2	75.0	3.42−3.48 (1H, m)
hexenoic moiety		3	78.0	3.42−3.48 (1H, m)	
1″	78.9		4	71.2	3.42−3.48 (1H, m)
2″	128.3	5.78 (1H, dd, 10.0, 2.5)	5	77.8	3.42−3.48 (1H, m)
3″	131.4	6.11 (1H, m)	6	62.5	3.85 (1H, dd, 12.0, 2.5)
4″	30.5	3.3−3.5 (2H, m)			3.70 (1H, dd, 12.0, 5.5)
5″	130.1				
6″	197.6				
7″	171.8				

Table 2. NMR Data (400/100 MHz, in DMSO-d_6) of **2**, δ in ppm and J in Hz

no.	δ_C	δ_H	no.	δ_C	δ_H
1	131.7		1″	102.8	4.61 (1H, d, 7.2)
2	148.5		2″	73.7	3.20 (m)
3	116.9	6.93 (1H, br d, 8.8)	3″	76.7	3.19 (m)
4	112.9	6.46 (1H, dd, 8.8, 2.9)	4″	69.6	3.24 (m)
5	152.2		5″	76.9	3.17 (m)
6	116.0	6.32 (1H, d, 2.9)	6″	60.7	3.57 (1H, dd, 11.6, 4.2)
7	29.4	3.74 (1H, d, 15.6)			3.48 (1H, dd, 11.6, 6.0)
		3.82 (1H, d, 15.6)	1‴	103.1	4.59 (1H, d, 7.2)
1′	125.3		2‴	73.7	3.20 (m)
2′	150.4		3‴	76.7	3.19 (m)
3′	114.1	6.84 (1H, s)	4‴	70.1	3.16 (m)
4′	130.9		5‴	77.2	3.24 (m)
5′	147.7		6‴	61.2	3.70 (1H, dd, 11.6, 5.6)
6′	119.2	6.81 (1H, s)			3.48 (1H, dd, 11.6, 6.0)
7′	58.4	4.53 (1H, d, 14.0)			
		4.39 (1H, d, 14.0)			

benzenoid system, *p*-disubstituted aromatic groups, two glucose units, and a hydroxymethylene group (CH_2OH-7′). The ^{13}C NMR spectrum (Table 2) showed the presence of another methylene ($-CH_2$-7). The glycosidation sites on the aromatic rings were established by HMBC correlations from H-1″ to C-2 and from H-1‴ to C-2′. In addition, HMBC correlations from H-7 to C-1, C-2, C-1′, C-2′, C-6, and C-6′ confirmed the linkage of two aromatic rings via C-7. Thus, the structure of **2** was identified as shown (itoside K).

HRESIMS analysis of compound **3** gave $[M + Na]^+$ at m/z 315.1045, indicating the molecular formula $C_{12}H_{20}O_8$ with an unsaturation index of 3. The ^1H NMR spectrum of **3** (Table 3) exhibited the presence of a glucopyranosyl moiety and a *cis*-alkene. In the ^{13}C NMR spectrum of **3** (Table 3), apart from the sugar moiety, two sp^2-hybridized carbons at δ 127.3 (C-2) and 134.3 (C-3), three sp^3-hybridized tertiary carbons connected to oxygen atoms (C-1, C-6, C-4), and one sp^3-hybridized secondary carbon (C-5) were also observed. HMBC correlations from both H-3 and H-1′ to C-1, from H-5 to C-1, C-3, C-6, and C-4, and from H-2 to C-1, C-6, and C-4 confirmed that compound **3** was a 1,4,6-trihydroxy-cyclohex-2-enoic-1-*O*-β-D-glycopyranoside. The relative configuration was resolved by the NOESY spectrum and was confirmed by X-ray crystallography (Figure 1). Consequently, compound **3** was established as 1β,4α,6β-trihydroxycyclohex-2-enoic-1-*O*-β-D-glycopyranoside (itoside L).

Compound **4** was obtained as an amorphous powder, and the molecular formula was determined to be $C_{12}H_{22}O_9$ by HRESIMS. Its ^1H, ^{13}C NMR (Table 3) and HMBC spectra were similar to those of **3**. The differences were that **4** had one less OH group than **3** and one more methylene group. The HMBC spectrum established the connectivity as shown in **4**, and NOESY correlations between H-1 and H-1′ and between H-1 and H-6 indicated that they were all on the same face of the molecule. Thus, the C-1 oxygen and the C-6 OH groups are on the β-face, and **4** was determined to be 1β,6β-dihydroxy-2-enoic-cyclohex-1-*O*-β-D-glucopyranoside (itoside M).

The molecular formula of compound **5** was established as $C_{39}H_{32}O_{14}$ by HRESIMS. The IR spectrum showed broad carbonyl absorption bands (approximately 1742 cm^{-1}). D-Glucose was detected by GC analyses after acid hydrolysis. The ^1H and ^{13}C NMR data (Table 4) indicated the presence of apigenin and acylated glycopyranosyl moieties. The HMBC spectrum (H-1″ and C-7) implied a partial structure in **5** similar to that of apigenin-7-*O*-β-D-glucopyranoside.[6] Comparison of their NMR data implied that there were two acylated positions on glucose in **5**, which was supported by the carbon chemical shift values and the $^1H-^1H$ COSY spectrum. The acylated positions (C-4″ and C-6″) were determined by HMBC correlations from H-4″ to C-1‴ and from H-6″ to C-1⁗. In the ^1H NMR spectrum of **5**, two additional *p*-disubstituted benzenoid groups and a cyclobutane [δ 42.9 (C-2‴), 43.5 (C-3‴), 45.5 (C-2⁗), 42.9 (C-3⁗)] moiety in combination with the two carbonyls mentioned above suggested the presence of a partial structure similar to that of *p*,*p*′-dihydroxy-μ-truxinic acid in **5**.[7] NOESY interactions observed between H-2‴/H-2⁗ and H-3‴/H-3⁗ confirmed that the two pairs of hydrogens were on the same side of the molecule. This implied that the two *p*-hydroxyphenyl rings are *cis* to each other. These assignments would then give two possible structures: one with two β-oriented *p*-hydroxy phenyl rings and another where one of them was α-oriented. However, the relative configuration of **5** was deduced finally to be as shown based on a lower minimum steric energy (E_{min}) using computer modeling. Although it is possible that a natural product is enzymetically biosynthesized in a high-energy conformation, in this case, a photochemically driven cycloaddition would result in the lowest energy product.[8] Thus, **5** was determined to be apigenin-7-*O*-β-D-

Table 3. NMR Data (500/125 MHz, in CD_3OD) of **3** and **4**, δ in ppm and J in Hz

	3		**4**	
no.	δ_C	δ_H	δ_C	δ_H
1	76.7	4.31 (1H, brs)	76.1	4.27 (1H, brs)
2	127.3	5.77 (1H, brd, 11.5)	125.7	5.74 (1H, m)
3	134.3	5.33 (1H, brd, 11.5)	132.2	5.85 (1H, ddd, 10.5, 3.5 1.5)
4	65.0	4.37 (1H, brs)	24.0	2.22, 2.03 (each 1H, m)
5	37.4	1.45 (1H, m), 2.04 (1H, m)	27.1	1.86, 1.69 (each 1H, m)
6	68.8	4.23 (1H, brt, 3.5)	69.0	3.92 (1H, ddd, 8.5, 3.0, 3.0)
1′	103.5	4.45 (1H, d, 8.0)	103.1	4.44 (1H, d, 8.0)
2′	75.0	3.34 (1H, m)	74.9	3.33 (1H, m)
3′	77.7	3.27 (1H, m)	77.1	3.28 (1H, m)
4′	71.4	3.38 (1H, m)	71.5	3.39 (1H, m)
5′	78.0	3.21 (1H, m)	77.9	3.22 (1H, m)
6′	62.5	3.84 (1H, dd, 12.0, 1.5)	62.5	3.85 (1H, dd, 10.0, 2.5)
		3.66 (1H, dd, 12.0, 5.0)		3.68 (1H, dd, 10.0, 6.0)

Table 4. NMR Data (500/125 MHz, in DMSO-d_6) of **5** and **6**, δ in ppm and J in Hz

	5		**6**	
no.	δ_C	δ_H	δ_C	δ_H
apigenin moiety				
2	164.2		164.2	
3	103.0	6.86 (1H, s)	103.0	6.82 (1H, s)
4	181.9		181.9	
5	161.9		161.9	
6	99.4	6.46 (1H, d, 2.0)	99.4	6.46 (1H, d, 2.0)
7	162.6		162.6	
8	94.7	6.80 (1H, d, 2.0)	94.7	6.80 (1H, d, 2.0)
9	156.8		156.8	
10	105.3		105.3	
1′	120.9		120.9	
2′, 6′	128.5	7.97 (2H, d, 8.0)	128.5	7.93 (2H, d, 8.0)
3′, 5′	115.9	6.97 (2H, d, 8.0)	115.9	6.92 (2H, d, 8.0)
4′	161.3		161.3	
glucose moiety				
1″	99.3	5.40 (1H, d, 7.5)	99.4	5.15 (1H, d, 7.5)
2″	72.8	3.45 (1H, t, 7.5)	72.9	3.82 (1H, t, 9.0)
3″	72.8	3.57 (1H, t, 9.5)	73.8	3.32 (1H, m)
4″	76.5	4.85 (1H, t, 9.5)	69.9	3.23 (1H, m)
5″	65.6	4.31 (1H, m)	76.2	3.34 (1H, m)
6″	64.7	3.90 (1H, dd, 11.5, 5.5)	61.8	3.44 (1H, dd, 7.0, 12.5)
		4.72 (1H, dd, 11.5, 2.5)		4.45 (1H, dd, 2.0, 12.5)
	4″-*p*-hydroxy-μ-truxinyl		2″-*p*-*E*-coumaroyl	
1‴	172.2		166.4	
2‴	42.9	3.80 (1H, dd, 10.0, 2.5)	113.7	6.33 (1H, d, 16.0)
3‴	43.5	4.20 (1H, dd, 10.0, 2.5)	144.8	7.47 (1H, d, 16.0)
4‴	128.2		124.8	
5‴, 9‴	129.2	6.95 (2H, d, 8.5)	130.0	7.36 (2H, d, 8.5)
6‴, 8‴	114.7	6.53 (2H, d, 8.5)	115.6	6.65 (2H, d, 8.5)
7‴	155.6		161.1	
6″-*p*-hydroxy-μ-truxinyl				
1⁗	170.7			
2⁗	45.5	4.02 (1H, dd, 10.0, 1.5)		
3⁗	42.9	4.18 (1H, dd, 10.0, 1.5)		
4⁗	128.3			
5⁗, 9⁗	128.6	6.72 (2H, d, 8.5)		
6⁗, 8⁗	114.6	6.47 (2H, d, 8.5)		
7⁗	155.5			

(4″,6″-*p*,*p*′-dihydroxy-μ-truxinyl)glucopyranoside, a new flavone glycoside (itoside N).

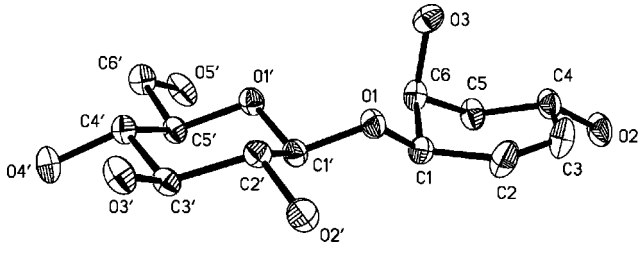


Figure 1. Single-crystal X-ray structure of **3**.

Echitin (**6**) was obtained as a yellow-brown, amorphous powder. Its molecular formula was deduced as $C_{30}H_{26}O_{12}$ by the sodium-adduct ion peak at m/z 601 $[M + Na]^+$ (ESIMS). The ^1H and ^{13}C NMR spectra were similar to those of apigenin-7-*O*-β-D-glucopyranoside. In these spectra, a *trans*-alkene, a *p*-disubstituted system, together with a carboxyl resonance at δ_C 166.4 (C-1‴), implied a *p*-*E*-coumaroyl moiety in **6**. HMBC correlation from H-2″ to C-1‴ determined its acetylation position to be C-2. Therefore, **6** was identified as echitin, whose structure has been reported previously.[2]

Also identified were 22 known compounds, salicin (**22**),[9] salirepin (**23**),[4] pyrocatechol-*O*-β-D-diglucopyranoside (**24**),[10] 3′-benzoylsalicin (**25**),[11] 2′-benzolypoliothyrsoside (**26**),[12] hydrangei-

Table 5. Anti-COX-2 Inhibitory Effects of Compounds from *I. orientalis*[a]

no.	I.R. (%)	no.	I.R. (%)	no.	I.R. (%)
1	3.3 ± 0.6	**13**	85.3 ± 0.6	**22**	7.7 ± 0.6
3	56.0 ± 1.0	**14**	64.7 ± 1.5	**23**	29.7 ± 1.5
5	67.3 ± 0.6	**15**[b]	−20.3 ± 0.6	**24**	54.3 ± 0.6
7	46.3 ± 0.6	**16**	53.0 ± 1.0	**25**	29.7 ± 2.0
8	61.0 ± 1.0	**17**	25.0 ± 1.0	**26**	35.7 ± 0.6
9	70.7 ± 2.3	**18**	31.3 ± 1.5	**27**	54.7 ± 0.6
10	17.3 ± 0.6	**19**	49.7 ± 1.5	**28**	41.0 ± 1.0
11	31.7 ± 0.6	**20**	46.3 ± 0.6		
12	62.0 ± 1.0	**21**	40.0 ± 1.0	NS398	85.3 ± 0.6

[a] Final concentration of all compounds (including NS398) is 1×10^{-5} mol/L, $n = 3$. [b] The tests were repeated several times, and the results were reproducible.

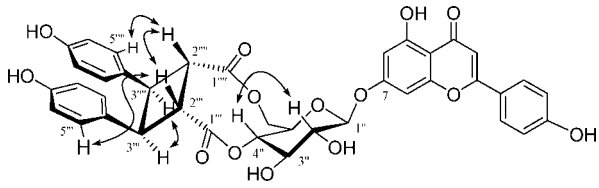


Figure 2. Relative configuration and NOESY data for **5**.

folin I (**27**),[13] vanilloloside (**28**),[14] syringin,[15] coniferin,[16] koaburoside,[17] tachioside,[18] isotachioside,[18] populin,[19] sasanquin,[20] 4-hydroxy-3,5-dimethoxybenzyl alcohol-4-*O*-*β*-D-glucopyranoside,[21] tricin-7-*O*-*β*-D-glucopyranoside,[22] apigenin,[23] chrysoeriol-7-*O*-*β*-D-glucopyranoside,[24] apigenin-8-*C*-*β*-D-glucopyranoside,[25] luteolin-8-*C*-*β*-D-glucopyranoside,[26] luteolin-7-*O*-*β*-D-glucopyranoside,[27] and apigenin-7-*O*-*β*-D-glucopyranoside,[6] by comparing their NMR data with references.

Itoside J (**1**) is a novel phenolic glycoside whose structure generally consists of two salirepin (**23**) units. Itoside N (**5**) is of special interest, as it is a rarely reported naturally occurring flavone glycoside truxinate ester. This is the first report of acylation on a single glucose moiety, although there are literature reports of truxinate and truxillate esters of alkaloids.[7]

Compounds **1**, **3**, **5**, and **7**−**28** were tested for anti-COX-2 activity. Compounds **3**, **5**, **10**, **12**−**14**, **16**, **19**, and **27** exhibited significant inhibition with inhibitory rates (I.R.) of 49.7−85.3% at 10 μM (Table 5). Compound **13** showed the highest inhibition rate (85.3%), which was equal to that of the positive control: NS398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methane sulfonamide).

Most of the phenolic glycosides assayed, including compounds **H-1** and **H-2** isolated from *Hydnocarpus annamensis,*[28] have gentisyl glycoside or salicin as basic units and possess a single glucose moiety, except for **1** and **15** (Figure 3). The structure−activity relationships may be summarized as follows. First, if the hydroxymethyl in the aglycone was esterified, such as in **8**−**14**, **16**, and **18**−**20**, the activity was enhanced compared with their counterparts. In general, compounds esterified by 2-hydroxybenzoic acid (**11**−**14**) were more active than those esterified by 1-hydroxy-oxocylohexenoic acid (**9**, **10**, **16**, and **19**). Second, anti-COX-2 activity was generally improved if benzoic acylation occurred on their glucose moieties, such as in compounds **26** (35.7%), **17** (25.0%), **7** (46.3%), and **H-1** (62.0%) compared with **23** (29.7%) and in **8** (61.0%), **9** (70.7%), **16** (53.0%), and **19** (49.7%) compared with **18** (31.3%). Compounds with this group at different positions exhibited various results. Activity also depended on whether the aglycone was esterified and on the nature of the ester group. Basically, when the aglycone was esterifed by 2-hydroxybenzoic acid, the increased order of activity with benzoic acid attached to the glucose unit was 4′ (**13**) > 6′ (**14**) > 3′ (**12**) > 2′ (**11**). When the alycone was esterified by 1-hydroxyoxocylohexenoic acid, the order of activity was 3′ (**9**) > 2′ (**16**) > 6′ (**19**) > 4′ (**10**) by benzoic acid attachment, but for those compounds without esterified groups on the aglycone the activity increased in the order 2′ (**7**) > 6′ (**17**) and at 3′ (**H-1**) > 4′ (**H-2**).

Compounds with an OH group at C-4 (**16**, **H-1**, and **23**) were generally more effective than those without an OH group at C-4 (**20**, **25**, and **22**). However, **1** showed very little inhibition against COX-2.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT4A digital micromelting point apparatus and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. NMR spectra were recorded in CD_3OD and DMSO-d_6 using Inova 500 and Bruker 400 MHz NMR spectrometers with tetramethylsilane as internal standard. HRFABMS was measured on an AutoSpec Ultima-TOF mass spectrometer and HRESIMS on a Bruker APEX IV FTMS mass spectrometer in positive ion mode. GC: Agilent 6890N (HP-5 capillary column (28 m × 0.32 mm, i.d.); detector, FID; temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2; flow rate, 40 mL/min). All solvents used were of analytical grade (Beijing Chemical and Industry Factory). Silica gel (200−300 mesh, Qingdao Mar. Chem. Ind. Co. Ltd.), Sephadex LH-20 gel (Pharmacia), and C_{18} reversed-phase silica gel (150−200 mesh, Merck, performed by applying a N_2 pressure of 0.12 MPa) were used for column chromatography.

Plant Material. The bark, twigs, and leaves of *Itoa orientalis* were collected in December 2004 from Xiashi Trees Park of the Chinese Academy of Forestry (CAF) in Guangxi Province, China, and authenticated by Mr. Maojing Yang, an engineer in Xiashi Trees Park, CAF. A voucher specimen was kept in the herbarium of Peking University Modern Research Centre for Traditional Chinese Medicine (IO20041205).

Extraction and Isolation. The dried bark (18 kg), twigs (22 kg), and leaves (18 kg) of *I. orientalis* was extracted twice with 80% EtOH (2 × 400 L, 2 h for each). After removal of the solvent under reduced pressure at 60 °C, the residue was suspended in H_2O (1.8 L) and extracted successively with $CHCl_3$ (2 × 5 L), EtOAc (2 × 5 L), and *n*-BuOH (2 × 5 L). The *n*-BuOH extract (520 g) of the bark and twigs was subjected to silica gel CC (14 × 130 cm) eluted with $CHCl_3$−MeOH (20:1−0:1) to yield fractions 1−7. The *n*-BuOH extract (280 g) of the leaves was subjected to silica gel CC (12 × 110 cm) eluted with $CHCl_3$−MeOH (12:1−0:1) to provide fractions A−K.

Chromatography of fraction 5 (38 g) on silica gel eluted with $CHCl_3$−MeOH (15:1−0:1) yielded fractions 5A−H. Chromatography of fraction 5H on silica gel eluted with EtOAc−MeOH (10:1), followed by Sephadex LH-20 (MeOH), and purified further using ODS (MeOH−H_2O, 3:7) afforded **4** (9 mg).

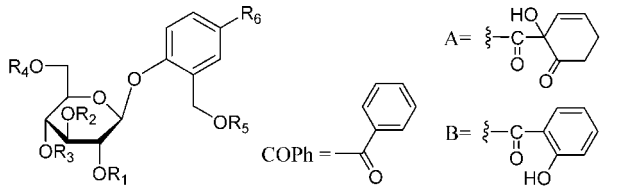
Fraction 6 (47 g) was subjected to silica gel CC eluted with EtOAc−MeOH−H_2O (10:1:0.1−0:1:0) to generate fractions 6I−XII. Fraction 6VI was further subjected to silica gel CC eluted with EtOAc−MeOH−H_2O (10:1:0.1) to afford fractions 6VIA−D. Purification of fraction 6VIB by Sephadex LH-20 followed by ODS (MeOH−H_2O, 1:1) afforded **1** (28 mg).

Fraction 7 (17 g) was subjected to silica gel CC eluted with EtOAc−MeOH−H_2O (8:2:0.5) to obtain fractions 7A−C. Fraction 7B (10.5 g) was rechromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (3:1:0.1) to yield fractions $7B_1$−B_4. Fraction $7B_4$ was subjected to Sephdex LH-20 (MeOH−H_2O, 7:3) followed by ODS (MeOH−H_2O, 2:8) to afford **2** (14 mg).

Fraction E (28 g) was subjected to silica gel CC eluted with EtOAc−MeOH (30:1) to obtain fractions E_1−E_3. Purification of fractions E_2 and E_3 on Sephadex LH-20 (MeOH) yielded **6** (21 mg) and **5** (52 mg), respectively.

Fraction J (33 g) was subjected to silica gel CC eluted with EtOAc−MeOH (10:1) to obtain fractions J_1−J_4. Fraction J_4 was rechromatographed on ODS eluted with MeOH−H_2O (3:7) followed by recrystallization from MeOH−H_2O (1:1) to obtain **3** (120 mg). For a detailed extraction protocol, see the Supporting Information.

Acid Hydrolysis of Compounds 1−6. Each compound (about 5 mg) was heated in 10% HCl−dioxane (1:1, 5 mL) at 80 °C for 4 h. After evaporation of dioxane, the solution was successively extracted with EtOAc (3 × 3 mL). The aqueous layer was neutralized with $NaHCO_3$ and concentrated. The solid residue from the aqueous layer was dissolved in anhydrous pyridine (100 μL). L-Cysteine methyl ester



No.	R_1	R_2	R_3	R_4	R_5	R_6	IR (%)
7	COPh	H	H	H	H	OH	46.3
8	COPh	H	H	COPh	A	OH	61.0
9	H	COPh	H	H	A	OH	17.3
10	H	H	COPh	H	A	OH	70.7
11	COPh	H	H	H	B	OH	31.7
12	H	COPh	H	H	B	OH	62.0
13	H	H	COPh	H	B	OH	85.3
14	H	H	H	COPh	B	OH	64.7
16	COPh	H	H	H	A	OH	53.0
17	H	H	H	COPh	H	OH	25.0
18	H	H	H	H	A	OH	31.3
19	H	H	H	COPh	A	OH	49.7
20	COPh	H	H	H	A	H	46.3
22	H	H	H	H	H	H	7.7
23	H	H	H	H	H	OH	29.7
25	H	COPh	H	H	H	H	29.7
26	COPh	H	H	COPh	H	OH	35.7
H-1[a]	H	COPh	H	H	H	OH	62.0
H-2[a]	H	H	COPh	H	H	OH	17.0

a: Isolated from *Hydnocarpus annamensis*

Figure 3. Phenolic glycosides from *I. orientalis* and *H. annamensis* and their inhibition rates against COX-2 at 10 μM.

hydrochloride (0.1 M, 200 μL) was added, and the mixture was warmed at 60 °C for 1 h. Then hexamethyldisilazane (HMDS)−chlorotrimethylsilane−pyridine (2:1:10, Acros Organics, Belgium) was added, and the mixture was warmed at 60 °C for an additional 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification: D-glucose derivative (t_R = 12.45 min).

Itoside J (1): yellow-green crystalline powder (MeOH−H_2O); mp 150−152 °C; $[\alpha]^{25}_D$ −42 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.92), 225 (4.71), 292 (4.33), 350.(3.96), 381 (2.28) nm; IR (KBr) ν_{max} 3404, 2922, 1704, 1708, 1597, 1495, 1452, 1280, 1212, 1070, 715 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD, 500/125 MHz), see Table 1; ESIMS *m/z*, 849 $[M + Na]^+$; HRESIMS *m/z*, 865.1942 $[M + K]^+$ (calcd for $C_{40}H_{43}O_{19}K$, 865.1952).

Itoside K (2): white, amorphous powder; $[\alpha]^{25}_D$ −24 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.71), 287 (3.85) nm; IR (KBr) ν_{max} 3420, 3384, 2920, 2887, 1628, 1509, 1218, 1212, 1080 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 500/125 MHz), see Table 2; ESIMS *m/z*, 609 $[M + Na]^+$, 604 $[M + NH_4]^+$; HRESIMS *m/z*, 587.19695 $[M + H]^+$ (calcd for $C_{26}H_{35}O_{15}$, 587.19705).

Itoside L (3): colorless crystals (MeOH−H_2O, 1:1); $[\alpha]^{25}_D$ −141 (*c* 0.60, MeOH); mp 210−212 °C; UV (MeOH) λ_{max} (log ϵ) 203 (3.67) nm; ^{1}H and ^{13}C NMR (CD_3OD, 500/125 MHz), see Table 3; HRESIMS *m/z*, 315.1045 $[M + Na]^+$ (calcd for $C_{12}H_{20}O_8Na$, 315.1050).

Itoside M (4): amorphous powder, $[\alpha]^{25}_D$ −35 (*c* 0.12, MeOH); ^{1}H and ^{13}C NMR (CD_3OD, 500/125 MHz), see Table 3; HRESIMS *m/z*, 299.1101 $[M + Na]^+$ (calcd for $C_{12}H_{22}O_9Na$, 299.1109).

Itoside N (5): yellow-brown, amorphous powder; $[\alpha]^{25}_D$ +10 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.36), 268 (4.14), 331(4.20) nm; IR (KBr) ν_{max} 3382, 2957, 1742, 1656, 1605, 1515, 1496, 1444, 1342, 1246, 1175, 1056, 837 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 500/125 MHz), see Table 4; HRESIMS *m/z*, 725.1887 $[M + H]^+$ (calcd for $C_{39}H_{33}O_{14}$, 725.1865).

Echitin (6): yellow-brown, amorphous powder; ^{1}H and ^{13}C NMR (DMSO-d_6, 500/125 MHz), see Table 4; ESIMS *m/z*, 601 $[M + Na]^+$.

Crystallographic Data for 3. $C_{12}H_{20}O_8$, M = 292.28, monoclinic, space group $P2_1$, a = 8.499(1) Å, b = 14.244(1) Å, c = 10.241(1) Å. V = 713.7(2) Å^3, Z = 2, d = 1.360 g/cm^3, crystal dimensions 0.15 × 0.40 × 0.50 mm^3 was used for measurement on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, $2\theta_{max}$ = 50.0), Mo Kα radiation. The total number of independent reflections measured was 1311, of which 1297 were observed ($|F|^2 \geq 2\sigma|F|^2$). R_F = 0.0402, R_w = 0.1077.

The crystal structure of **3** was solved by the direct method using SHELX-97 and using difference Fourier techniques, refined by the program NOMCSDP8 and full-matrix least-squares calculations. Crystallographic data for this structure have been deposited in the Cambridge Crystallographic Data Centre (CCDC 643405). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving-.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk].

Inhibition of COX-2 Assay. The COX-2 assay described by Duan[29] was used for the measurement of COX-2 inhibitory activity. In a 78 μL reaction system, Tris-HCl buffer (0.1 M, pH = 8.0), 4.4% heme final concentration, and COX-2 (Cayman, Ann Arbor, MI) were added successively. After 5 min preincubation at 25 °C, TMPD and arachidonic acid (Cayman) were added. NS398 (Cayman) was used as positive control, with its inhibitory rate at 85.3% at 10 μM. After a second 5 min preincubation at 25 °C, enzyme activity was monitored on a Greiner 384 microplate reader (TECAN Safire2, Switzerland) by following the rate (change in OD/min) of I.R.% = (total absorption − samples' absorption)/total absorption × 100% at 590 nm. All assays were conducted in triplicate.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1**, **2**, and **5** and CIF file for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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