Tetrasaccharide Multi-Esters and Xanthone Glycosides from the Roots of *Polygala wattersii*

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Ten new tetrasaccharide multi-esters, watteroses A−J (**1**−**10**), and two new xanthone glycosides, wattersiixanthones A (**11**) and B (**12**), were isolated from the roots of *Polygala wattersii*, together with 11 known compounds (10 oligosaccharide multi-esters and a xanthone glycoside). The structures of new compounds were elucidated on the basis of chemical and spectroscopic evidence.

In the course of a research program on the oligosaccharide esters from *Polygala* species,[1] we investigated *P. wattersii* Hance (Polygalaceae). *P. wattersii* is widely distributed in the People's Republic of China, and its root is used as a tonic in traditional medicine. No previous investigation has been reported on this plant. We now report the isolation and structure elucidation of 10 new tetrasaccharide multi-esters, watteroses A−J (**1**−**10**), and two new xanthone glycosides, wattersiixanthones A (**11**) and B (**12**). Ten known oligosaccharide multi-esters isolated from this plant were identified by comparison of the spectral data with reported data, as reiniose G (**13**),[2] fallaxose C (**14**),[3] reiniose H (**15**),[2] senegose F (**16**),[4] senegose G (**17**),[4] reiniose A (**18**),[3] 6-*O*-benzoyl-3′-*O*-3,4,5-trimethoxycinnamoyl-sucrose (**19**),[5] 6-*O*-feruoyl-3′-sinapoyl-sucrose (**20**),[5] 3′-*O*-feruloyl-6-*O*-sinapoyl-sucrose (**21**), [5] 3′,6-di-*O*-sinapoyl-sucrose (**22**),[5] and a known xanthone glycoside identified as wubangziside B (**23**).[6] See Chart 1.

Results and Discussion

Air-dried roots of *P. wattersii* were extracted with MeOH under reflux. The MeOH extract was suspended in H_2O and extracted with ether. The H_2O layer was adsorbed on a porous polymer gel (Diaion HP-20) column. The material was eluted with 50% aqueous MeOH, 70% aqueous MeOH, and MeOH, successively. The 70% aqueous MeOH eluate was chromatographed on a Si gel column using $CHCl_3$−MeOH−H_2O, and selected fractions were then subjected to preparative HPLC, using a reversed-phase (ODS) column, which led to the isolation of 15 tetrasaccharide multi-esters (**1**−**10** and **13**−**17**), five sucrose esters (**18**−**22**), and three xanthone glycosides (**11**, **12**, and **23**).

Watterose A (**1**) was isolated as an amorphous powder. The positive mode FABMS revealed a quasimolecular ion peak at *m*/*z* 1157 $[M + Na]^+$ consistent with a molecular formula of $C_{54}H_{62}O_{28}$. On acid hydrolysis, **1** gave D-glucose and D-fructose[7] in the ratio 3:1. Alkaline hydrolysis afforded a mixture composed of benzoic, *p*-coumaric, and ferulic acid. In the ^1H NMR spectrum of **1**, acetyl, benzoyl, *p*-coumaroyl, and feruloyl signals were observed (See Tables 1 and 2). HOHAHA difference spectra on irradiation at each anomeric proton signal and H-3 of the fructosyl moiety and ROE experiments involving irradiation at each anomeric proton signal enabled us to assign all proton signals of the Glc1, Glc2, Glc3, and Fru moieties. The sugar and acyl residue linkages were assigned from ROE and HMBC. In the ROE difference spectra of **1**, when the proton signals at δ 4.60 (1H, d, J = 7.5 Hz, H-1 of Glc2) and 4.47 (1H, d, J = 8 Hz, H-1 of Glc3) were irradiated, ROEs were observed at δ 3.77 (1H, dd, J = 10, 3.5 Hz, H-2 of Glc1) and 3.97 (1H, dd, J = 10, 10 Hz, H-3 of Glc1), respectively. In the HMBC spectrum $^nJ_{C-H}$ correlations were observed between the *p*-coumaroyl carbonyl carbon signal at δ 168.1 and the proton signal at δ 5.00 (1H, dd, J = 10, 10 Hz, H-4 of Glc1); the feruloyl carbonyl carbon signals at δ 168.4 and the proton signal at δ 4.22, 4.73 (each 1H, d, J = 12 Hz, H-1 of Fru); the benzoyl carbonyl carbon signal at δ 167.4 and the proton signal at δ 5.72 (1H, d, J = 8 Hz, H-3 of Fru); the acetyl carbonyl carbon signal at δ 172.7 and the proton signals at δ 3.96, 4.06 (each 1H, overlapped, H-6 of Glc3); the carbon signal at δ 79.3 (C-3 of Glc1) and the proton signal at δ 4.47 (H-1 of Glc3); the carbon signal at δ 81.5 (C-2 of Glc1) and the proton signal at δ 4.60 (H-1 of Glc2); and the carbon signal at δ 103.9 (C-2 of Fru) and the proton signal at δ 5.84 (1H, d, J = 3.5 Hz, H-1 of Glc1). These data led us to assign the structure of watterose A as **1**.

The FABMS of watterose B (**2**) gave a quasimolecular ion peak at *m*/*z* 1185 $[M + Na]^+$, 28 mass units higher than that of **1**, and ^{13}C NMR data were consistent with a molecular formula of $C_{53}H_{62}O_{29}$. The ^1H and ^{13}C NMR spectra of **2** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, a *p*-coumaroyl, a caffeoyl, and two acetyl moieties. The NMR spectra were similar to those of **1**, except for the presence of a caffeoyl residue instead of a feruloyl residue, and C-6 of Glc1 was acetylated. The position of each acyl residue was defined by the HMBC spectrum. These data led us to assign the structure of watterose B as **2**.

The FABMS of watterose C (**3**) gave a quasimolecular ion peak at *m*/*z* 1143 $[M + Na]^+$, 13 mass units higher than that of **1**, and ^{13}C NMR data were consistent with a molecular formula of $C_{51}H_{60}O_{28}$. The ^1H and ^{13}C NMR spectra of **3** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, a *p*-coumaroyl, a caffeoyl, and an acetyl moieties. The NMR spectra were similar to those of **1**, except for the presence of a caffeoyl residue instead of a feruloyl residue. These data led us to assign the structure of watterose C as **3**.

The FABMS of watterose D (**4**) gave a quasimolecular ion peak at *m*/*z* 1101 $[M + Na]^+$, 42 mass units lower

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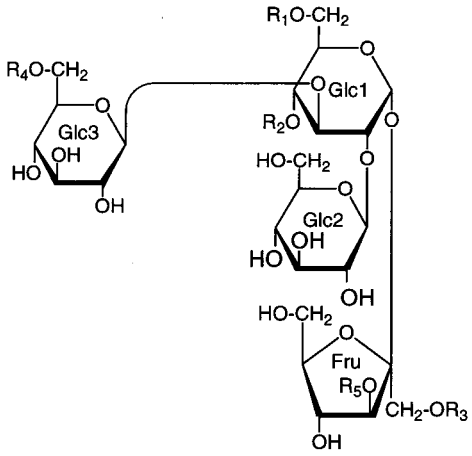
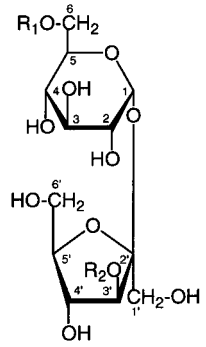
Chart 1

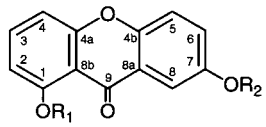


	R_1	R_2	R_3	R_4	R_5
1	H	**B**	**C**	**A**	**E**
2	**A**	**B**	**D**	**A**	**E**
3	H	**B**	**D**	**A**	**E**
4	H	**B**	**D**	H	**E**
5	**A**	**C**	**D**	**A**	**E**
6	H	**C**	**D**	**A**	**E**
7	**A**	**D**	**D**	**A**	**E**
8	**A**	**B**	**E**	**A**	**E**
9	H	**D**	**E**	**A**	**E**
10	**D**	H	**D**	**A**	**E**
13	**A**	**B**	**B**	**A**	**E**
13a	H	H	H	H	H
14	H	**B**	**B**	**A**	**E**
15	**A**	**B**	**C**	**A**	**E**
16	**A**	**C**	**C**	**A**	**E**
17	H	**C**	**C**	**A**	**E**



	R_1	R_2
18	**C**	**F**
19	**E**	**F**
20	**C**	**G**
21	**G**	**C**
22	**G**	**G**

A : acetyl
B : *p*-coumaroyl
C : feruloyl
D : caffeoyl
E : benzoyl
F : 3,4,5-trimethoxy cinnamoyl
G : sinapoyl



	R_1	R_2
11	Me	Glc —(6)— Api
12	Me	Glc
23	H	Glc —(6)— Api

than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $C_{49}H_{58}O_{27}$. The ^1H and ^{13}C NMR spectra of **4** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, a *p*-coumaroyl, and a caffeoyl moieties. The NMR spectra were similar to those of **3**, except that **4** had no acetyl residue. These data led us to assign the structure of watterose D as **4**.

The FABMS of watterose E (**5**) gave a quasimolecular ion peak at m/z 1215 $[M + Na]^+$, 30 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $C_{54}H_{64}O_{30}$. The ^1H and ^{13}C NMR spectra of **5** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, a feruloyl, a caffeoyl, and two acetyl moieties. The NMR spectra were similar to those of **2**, except for the presence of a feruloyl residue instead of a *p*-coumaroyl residue. These data led us to assign the structure of watterose E as **5**.

The FABMS of watterose F (**6**) gave a quasimolecular ion peak at m/z 1173 $[M + Na]^+$, 30 mass units higher than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $C_{53}H_{62}O_{29}$. The ^1H and ^{13}C NMR spectra of **6** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, a feruloyl, a caffeoyl, and an acetyl moieties. The NMR spectra were similar to those of **3**, except for the presence of a feruloyl residue instead of a *p*-coumaroyl residue. These data led us to assign the structure of watterose F as **6**.

The FABMS of watterose G (**7**) gave a quasimolecular ion peak at m/z 1201 $[M + Na]^+$, 16 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $C_{53}H_{62}O_{30}$. The ^1H and ^{13}C NMR spectra of **7** showed that this compound was composed of a D-fructose, three D-glucose, a benzoyl, two caffeoyl, and two acetyl moieties. The NMR spectra were similar to those of **2**, except for the presence of a caffeoyl residue instead of a *p*-coumaroyl residue. These data led us to assign the structure of watterose G as **7**.

The FABMS of watterose H (**8**) gave a quasimolecular ion peak at m/z 1127 $[M + Na]^+$, 58 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $C_{51}H_{60}O_{25}$. The ^1H and ^{13}C NMR spectra of **8** showed that this compound was composed of a D-fructose, three D-glucose, two benzoyl, a *p*-coumaroyl, and two acetyl moieties. The NMR spectra were similar to those of **2**, except for the presence of a benzoyl residue

Table 1. ^{1}H NMR Data of **1**−**10** in CD_3OD at 35 °C[a]

	1	2	3	4	5	6	7	8	9	10
Sugar moiety										
Glc1-1	5.84 d (3.5)	5.86 d (3.5)	5.85 d (3.5)	5.83 d (3.5)	5.86 d (3.5)	5.87 d (3.5)	5.85 d (3.5)	5.90 d (3.5)	5.87 d (3.5)	5.78 d (3)
2	3.77 dd (10, 3.5)	3.81 dd (10, 3.5)	3.79 dd (10, 3.5)	3.78 dd (10, 3.5)	3.81 dd (10, 3.5)	3.81 dd (10, 3.5)	3.80 dd (9, 3.5)	3.82 dd (10, 3.5)	3.78 dd (9, 3.5)	3.72 dd (10, 3)
3	3.97 dd (10, 10)	3.95 dd (10, 10)	3.97[a]	4.01 dd (10, 10)	3.97 dd (10, 10)	3.96 dd (10, 10)	3.95 dd (9, 9)	3.97[b]	3.98 dd (9, 9)	3.68 dd (8, 8)
4	5.00 dd (10, 10)	5.00 dd (10, 9)	5.01 dd (10, 10)	5.00 dd (10, 10)	5.01 dd (10, 9)	5.04 dd (10, 9)	5.00 dd (9,9)	5.01 dd (9, 9)	5.00 dd (9, 9)	3.47 dd (8, 8)
5	4.21 m	4.38 m	4.23 m	4.22 m	4.39 m	4.24 m	4.39 m	4.42 m	4.23 m	4.26 m
6	3.68 dd (12, 2)	4.18 dd (12, 2.5)	3.70 dd (12, 2.5)	3.69 dd (12, 2.5)	4.18 dd (12, 2.5)	3.72 dd (12, 2)	4.18[b]	4.20 dd (12, 3)	3.69 dd (12, 2)	4.59 dd (12, 2)
	3.56 dd (12, 5)	4.13 dd (12, 5.5)	3.58 dd (12, 5)	3.57[b]	4.13 dd (12, 5.5)	3.60 dd (12, 5)	4.14 dd (12, 5.5)	4.14 dd (12, 5.5)	3.56 dd (12, 5.5)	4.32 m
Glc2-1	4.60 d (7.5)	4.59 d (7.5)	4.61 d (7.5)	4.59 d (7)	4.60 dd (7)	4.63 d (7)	4.59 d (7.5)	4.60 d (7.5)	4.60 d (7.5)	4.53 d (7.5)
2	3.32[b]	3.32[b]	3.33[b]	3.31[b]	3.32[b]	3.34[b]	3.32[b]	3.32[b]	3.32[b]	3.32[b]
3	3.32[b]	3.32[b]	3.33[b]	3.31[b]	3.32[b]	3.34[b]	3.32[b]	3.32[b]	3.32[b]	3.32[b]
4	3.32[b]	3.32[b]	3.33[b]	3.31[b]	3.32[b]	3.34[b]	3.32[b]	3.32[b]	3.32[b]	3.32[b]
5	3.32[b]	3.32[b]	3.33[b]	3.31[b]	3.32[b]	3.34[b]	3.32[b]	3.32[b]	3.32[b]	3.32[b]
6	3.93[b]	3.94[b]	3.94[b]	3.72[b]	3.93[b]	3.97[b]	3.93[b]	3.94[b]	3.92[b]	3.92[b]
	3.73 dd (12, 6)	3.72 dd (11.5, 5.5)	3.73 dd (12, 5)	3.93 d (12)	3.72 dd (11.5, 5.5)	3.77[b]	3.72 dd (12, 5.5)	3.71 dd (12, 5.5)	3.72 dd (12, 5.5)	3.71[b]
Glc3-1	4.47 d (8)	4.49 d (8)	4.47 d (8)	4.42 d (8)	4.49 d (8)	4.51 d (7.5)	4.48 d (8)	4.46 d (8)	4.44 d (8)	4.24 d (7.5)
2	3.01 dd (8, 9)	3.01 dd (8, 9)	3.03 dd (8, 9)	3.01[b]	3.01 dd (9, 8)	3.05 dd (8, 7.5)	3.01 dd (8, 8)	3.01 dd (8, 9)	3.01 dd (8, 9)	3.17 m
3	3.19[b]	3.19[b]	3.21[b]	3.19[b]	3.18[b]	3.22[b]	3.19[b]	3.19[b]	3.16 dd (9, 9)	3.19 m
4	3.19[b]	3.19[b]	3.21[b]	3.19[b]	3.20[b]	3.22[b]	3.19[b]	3.19[b]	3.21 dd (9, 9)	3.24 m
5	3.07[b]	3.10[b]	3.09[b]	3.04[b]	3.10 m	3.11 m	3.09 m	3.06 m	3.05 m	3.22 m
6	4.06[b]	4.06[b]	4.07 dd (12, 4)	4.06 br d (12)	4.06[b]	4.11 dd (12, 4)	4.07 dd (11, 3.5)	4.08[b]	4.09 dd (11, 3.5)	4.35 m
	3.96[b]	3.96[b]	3.96[b]	3.45 dd (12, 5.5)	4.00[b]	4.01[b]	3.95 br d (11)	3.98[b]	3.95 br d (11)	4.12 m
Fru-1	4.22 d (12)	4.19 d (12)	4.22 d (12)	4.19 d (12)	4.19 d (12)	4.24 d (12)	4.19 d (12)	4.32 d (12)	4.33 d (12)	4.14 d (12)
	4.73 d (12)	4.71 d (12)	4.74 d (12)	4.74 d (12)	4.71 d (12)	4.76 d (12)	4.71 d (12)	4.85 d (12)	4.86 d (12)	4.71 d (12)
3	5.72 d (8)	5.72 d (8)	5.72 d (8)	5.72 d (8)	5.72 d (8)	5.74 d (8)	5.72 d (8)	5.79 d (8)	5.78 d (8)	5.73 d (8)
4	4.51 dd (8, 8)	4.43 dd (8.5, 8.5)	4.53 dd (8, 8)	4.52 dd (8, 8)	4.43 dd (8.5, 8.5)	4.55 dd (8, 8)	4.44 dd (8, 8)	4.46 t (8)	4.53 dd (8, 8)	4.50 dd (8, 8)
5	4.04[b]	4.07[b]	4.04[b]	4.03[b]	4.07[b]	4.07m	4.06[b]	4.08 m	4.04 m	4.06[b]
6	3.87[b]	3.86[b]	3.87[b]	3.87[b]	3.86[b]	3.90[b]	3.83[b]	3.89 m	3.88[b]	3.93 m
	3.87[b]	3.86[b]	3.87[b]	3.87[b]	3.86[b]	3.90[b]	3.83[b]	3.85 m	3.86[b]	3.83 dd (12, 2.5)
Ac (R_1)		2.06 s			2.06 s	2.06 s	2.06 s	2.07 s		
Ac (R_4)	1.63 s	1.63 s	1.63 s		1.60 s	1.65 s	1.65 s	1.63 s	1.66 s	2.05 s
acid										
(at C-4 of Glc1)β	6.22 d (16)	6.22 d (16)	6.23 d (16)	6.30 d (16)	6.25 d (15, 5)	6.30 d (16)	6.17 d (16)	6.22 d (16)	6.18 d (16) (at C-6 of Glc1)	6.34 d (16)
γ	7.56 d (16)	7.56 d (16)	7.56 d (16)	7.54 d (16)	7.56 d (12)	7.61 d (16)	7.50 d (2)	7.56 d (16)	7.50 d (16)	7.58 d (16)
2	7.44 d (8.5)	7.45 d (8)	7.45 d (8.5)	7.49 d (8.5)	7.21 d (2)	7.24 d (2)	7.05 d (2)	7.45 d (8)	7.05 d (2)	7.06 d (2)
3	6.85 d (8.5)	6.85 d (8)	6.86 d (8.5)	6.85 d (8.5)				6.85 d (8)	6.94 dd (8, 2)	6.77 d (8)
5	6.85 d (8.5)	6.85 d (8)	6.86 d (8.5)	6.85 d (8.5)	6.85 d (8)	6.88 d (8)	6.82 d (8)	6.85 d (8)	6.82 d (8)	6.89 dd (8, 2)
6	7.44 d (8.5)	7.44 d (8)	7.45 d (8.5)	7.49 d (8.5)	7.06 dd (8, 2)	7.09 dd (8, 2)	6.94 dd (8, 2)	7.45 d (8)		6.29 d (16)
(at C-1 of Fru)β	6.41 d (16)	6.31 d (16)	6.32 d (16)	6.32 d (16)	3.95 s	3.98 s				7.59 d (16)
γ	7.68 d (16)	7.62 d (16)	7.63 d (16)	7.63 d (16)	6.31 d (16)	6.34 d (16)	6.31 d (16)		8.10 dd (7.5, 1.5)	7.03 d (2)
2	7.20 d (2)	7.05 d (2)	7.06 d (2)	7.05 d (2)	7.62 d (16)	7.66 d (16)	7.62 d (16)		7.47 t (7.5)	
5	6.81 d (8)	6.79 d (8)	6.79 d (8)	6.79 d (8.5)	7.05 d (2)	7.08 d (2)	7.05 d (2)	8.10 dd (7.5, 1.5)	7.61 tt (7.5, 1.5)	6.77 d (8)
6	7.03 dd (8, 2)	6.91 dd (8, 2)	6.92 dd (8, 2)	6.93 dd (8.5, 2)				7.46 t (7.5)	8.10 dd (7.5, 1.5)	6.89 dd (8, 2)
OMe	3.91 s							7.61 tt (7.5, 1.5)	8.18 dd (7.5, 1.5)	8.15 dd (7, 1)
(at C-3 of Fru)2	8.20 br d (7.5)	8.17 dd (7.5, 1.5)	8.19 dd (8.5, 1.5)	8.18 dd (8.5, 2)	6.78 d (8)	6.82 d (8)	6.76 d (8)	7.46 t (7.5)	7.59 t (7.5)	7.54 t (7)
3	7.59 t (8)	7.58 t (7.5)	7.58 t (8)	7.58 t (8.5)	6.91 dd (8, 2)	6.95 dd (8, 2)	6.92 dd (8, 2)	8.10 dd (7.5, 1.5)	7.72 tt (7.5, 1.5)	7.66 tt (7, 1)
4	7.70 tt (7,1)	7.70 tt (7.5, 1.5)	7.69 tt (8,1.5)	7.64 tt (8,1.5)	8.17 dd (8, 1.5)	8.23 dd (7.5, 1.5)	8.18 dd (8, 1.5)	8.16 dd (7.5, 1.5)	7.59 t (7.5)	7.54 t (7)
5	7.59 t (8)	7.58 t (7.5)	7.58 t (8)	7.58 t (8.5)	7.59 t (8)	7.63 t (7.5)	7.59 t (8)	7.58 t (7.5)	8.18 dd (7.5, 1.5)	8.15 dd (7, 1)
6	8.20 br d (7.5)	8.17 dd (7.5, 1.5)	8.19 dd (8.5, 1.5)	8.18 dd (8.5, 2)	7.69 tt (8, 1)	7.72 tt (7.5, 1.5)	7.72 dd (7.5, 1.5)	7.70 tt (7.5, 1.5)		

[a] Assigned with the aid of HOHAHA difference, ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Overlapped with other signals.

Table 2. ^{13}C NMR Data of **1**−**10** in CD_3OD at 35 °C

	1	2	3	4	5	6	7	8	9	10
sugar moiety										
Glc1-1	93.2	92.9	93.1	93.2	93.0	93.2	92.9	93.0	93.2	93.0
2	81.5	81.3	81.4	81.1	81.4	81.5	81.3	81.4	81.6	80.2
3	79.3	79.0	79.2	79.8	79.0	79.2	79.0	79.0	79.2	85.0
4	70.4	70.5	70.3	71.0	70.5	70.4	70.5	70.5	70.4	70.6
5	72.3	69.7	72.2	72.2	69.7	72.3	69.6	69.7	72.3	71.8
6	63.2	64.4	62.2	62.1	64.4	62.2	64.3	64.4	62.2	65.0
Glc2-1	105.4	105.4	105.3	105.5	105.4	105.4	105.4	105.4	105.5	105.8
2	75.5	75.3	75.2	75.3	75.3	75.3	75.3	75.3	75.3	75.2
3	78.5	78.5	78.4	78.4	78.5	78.5	78.5	78.5	78.5	78.3
4	71.7	71.6	71.6	71.7	71.7	71.7	71.6	71.7	71.7	71.5
5	77.9	77.9	77.8	77.9	77.9	77.9	78.5	77.9	77.9	77.1
6	62.2	63.1	63.0	63.0	63.1	63.2	63.0	63.1	63.2	63.1
Glc3-1	104.5	104.5	104.4	104.7	104.4	104.5	104.5	104.5	104.5	105.2
2	75.7	75.5	75.4	75.6	75.5	75.5	75.5	75.5	75.5	75.2
3	78.5	78.5	78.5	78.4	78.5	78.5	77.8	78.5	78.5	78.3
4	71.1	71.1	71.0	71.7	71.1	71.1	71.0	71.1	71.0	71.5
5	74.7	74.7	74.6	74.9	74.7	74.7	74.7	74.7	74.7	77.1
6	64.5	64.4	64.4	62.9	64.4	64.4	64.3	64.4	64.3	63.1
Fru-1	66.0	65.9	65.9	65.8	65.8	65.9	65.9	66.4	66.4	65.7
2	103.9	104.0	103.8	103.9	104.0	103.9	103.9	104.0	103.8	103.8
3	80.2	80.1	80.1	80.0	80.1	80.2	80.1	80.2	80.2	80.0
4	73.6	74.0	73.6	73.6	74.0	73.6	74.0	74.0	73.6	74.0
5	84.6	84.7	84.5	84.6	84.7	84.5	84.7	84.7	84.6	84.5
6	63.1	63.7	63.1	63.2	63.8	63.1	63.8	63.8	63.3	64.1
Ac (R_1)		172.5			172.5		172.5	172.5		
		20.8			20.8		20.7	20.8		
Ac (R_4)	172.7	172.6	172.7		172.6	172.6	172.8	172.6	172.8	172.7
	20.5	20.5	20.5		20.5	20.5	20.5	20.5	20.5	20.5
acid									(at C-6 of Glc1)	
(at C-4 of Glc1)α	168.1	167.9	168.1	168.3	167.9	168.1	167.9	167.9	168.1	169.1
β	115.2	115.0	115.2	115.2	115.4	115.5	115.0	115.0	115.2	115.0
γ	146.6	146.8	146.7	146.9	147.1	146.9	147.2	146.8	147.0	147.3
1	127.1	127.1	127.1	127.2	127.6	127.6	127.6	127.0	127.7	127.8
2	131.3	131.3	131.3	131.3	111.8	111.7	115.5	131.3	115.5	115.3
3	117.0	117.0	117.0	116.9	149.5	149.5	146.9	117.0	146.9	146.8
4	161.4	161.5	161.3	161.4	150.9	150.9	149.6	161.5	149.8	149.6
5	117.0	117.0	117.0	116.9	116.5	116.5	116.6	117.0	116.6	116.5
6	131.3	131.3	131.3	131.3	124.5	124.5	123.0	131.3	122.9	123.1
OMe					56.5	56.5				
(at C-1 of Fru)α	168.4	168.5	168.5	168.5	168.5	168.5	168.4	167.4	167.4	168.5
β	115.3	114.8	114.8	114.8	114.8	114.8	114.8			115.3
γ	147.3	147.4	147.4	147.4	146.8	147.4	147.4			147.3
1	127.7	127.8	127.7	127.8	127.8	127.8	127.7	130.9	131.0	127.8
2	111.8	115.3	115.3	115.3	115.3	115.3	115.3	130.7	130.7	115.3
3	149.4	146.8	146.6	146.8	146.8	146.8	146.8	129.6	129.6	146.8
4	150.8	149.7	149.6	149.6	149.7	149.7	149.7	134.4	134.4	149.6
5	116.5	116.5	116.5	116.5	116.5	116.5	116.5	129.6	129.6	116.5
6	124.3	123.0	123.0	123.1	123.1	123.1	123.0	130.7	130.7	123.1
OMe	56.6									
(at C-3 of Fru)α	167.4	167.3	167.3	167.4	167.3	167.4	167.3	167.3	167.3	167.4
1	131.1	130.9	131.0	130.9	131.1	131.1	130.9	131.2	131.2	130.9
2	131.1	131.0	131.1	131.0	131.1	131.1	130.9	131.0	131.1	131.1
3	129.9	129.9	129.8	129.9	129.9	129.9	130.0	129.9	129.9	129.9
4	134.8	134.9	134.8	134.7	134.8	134.8	135.0	134.9	134.9	134.8
5	129.9	129.9	129.8	129.9	129.9	129.9	130.0	129.9	129.9	129.9
6	131.1	131.0	131.1	131.0	131.1	131.1	130.9	131.0	131.1	131.1

instead of a caffeoyl residue. These data led us to assign the structure of watterose H as **8**.

The FABMS of watterose I (**9**) gave a quasimolecular ion peak at m/z 1101 $[M + Na]^+$, 42 mass units higher than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $C_{47}H_{58}O_{27}$. The ^1H and ^{13}C NMR spectra of **9** showed that this compound was composed of a D-fructose, three D-glucose, two benzoyl, a caffeoyl, and an acetyl moieties. The NMR spectra were similar to those of **3**, except for the presence of a benzoyl residue instead of a caffeoyl residue. These data led us to assign the structure of watterose I as **9**.

The FABMS of watterose J (**10**) gave a quasimolecular ion peak at m/z 1137 $[M + H]^+$, and ^{13}C NMR data were consistent with a molecular formula of $C_{51}H_{60}O_{29}$. On acid hydrolysis **10** gave D-glucose and D-fructose in the ratio 3:1. Alkaline hydrolysis afforded an acid mixture composed of benzoic and caffeic acid. In the ^1H NMR spectrum of **10**, acetyl, benzoyl, and caffeoyl signals were observed (see Tables 1 and 2). All proton signals of the Glc1, Glc2, Glc3, and Fru moieties were assigned by the HOHAHA spectrum. The sugar and acyl residue linkages were assigned from HMBC. In the HMBC spectrum, $^nJ_{C-H}$ correlations were observed between the caffeoyl carbonyl carbon signal at δ 169.1 and the proton signals at δ 4.32 (1H, overlapped) and 4.59 (1H, dd, J = 12, 12 Hz) due to H_2-6 of Glc1; another caffeoyl carbonyl carbon signal at δ 168.5 and the proton signals at δ 4.14, 4.71 (each 1H, d, J = 12 Hz) due to H_2-1 of Fru; the benzoyl carbonyl carbon signal at δ 167.4 and the proton signal at δ 5.73 (1H, d, J = 8 Hz) due to

H-3 of Fru; the carbon signal at δ 85.0 (C-3 of Glc1) and the proton signal at δ 4.24 (1H, d, J = 7.5 Hz) due to H-1 of Glc3; the carbon signal at δ 103.8 (C-2 of Fru) and the proton signal at δ 5.78 (1H, d, J = 3 Hz) due to H-1 of Glc1; and the carbon signal at δ 80.2 (C-2 of Glc1) and the proton signal at δ 4.53 (1H, d, J = 5.5 Hz) due to H-1 of Glc2. These data led us to assign the structure of watterose J as **10**.

Wattersiixanthone A (**11**) was isolated as an amorphous powder. The positive mode FABMS revealed quasimolecular ion peaks at m/z 537 $[M + H]^+$ and 559 $[M + Na]^+$ consistent with a molecular formula of $C_{25}H_{28}O_{13}$. The NMR spectra were similar to those of wubangziside B (**23**),[6] except for the presence of methoxyl group. From the HMBC spectrum, the methoxyl group was bound at the carbon at δ 160.2 (C-1 of xanthone). These data led us to assign the structure of wattersiixanthone A as **11**.

Wattersiixanthone B (**12**) was isolated as an amorphous powder. The positive-mode FABMS revealed quasimolecular ion peaks at m/z 405 $[M + H]^+$ and 427 $[M + Na]^+$ consistent with a molecular formula of $C_{20}H_{20}O_9$. The NMR spectra were similar to those of **11**, except for lacking of apiose residue. These data led us to assign the structure of wattersiixanthone B as **12**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on Hitachi U-3410 spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL α-400 FT-NMR spectrometer with TMS as internal standard. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulse-field gradient. Positive-mode FABMS were recorded on a JEOL JMS-SX102 spectrometer, using a *m*-nitrobenzyl alcohol matrix. GC was carried out with Hitachi G-3000 gas chromatograph. HPLC was performed using a JASCO System 800.

Plant Material. *P. wattersii* was collected in May 1996, Sichuan, People's Republic of China. The plant was identified by Prof. Zhaoguang Liu, Chengdu Institute of Biology, Academia Sinica, People's Republic of China, and the voucher specimen (no. 960516) has been deposited in the herbarium, School of Pharmaceutical Sciences, University of Shizuoka.

Extraction and Isolation. The dried roots of *P. wattersii* (1.85 kg) were extracted twice with MeOH under reflux. After evaporation of the solvent under reduced pressure, the MeOH extract was suspended in H_2O and extracted with ether. The H_2O layer was subjected to porous polymer gel Mitsubishi Diaion HP-20 column (9 × 41 cm). The adsorbed material was eluted with 50% aqueous MeOH, 70% aqueous MeOH, and MeOH successively, after washing with H_2O. The 70% aqueous MeOH eluate (20.8 g) was chromatographed on a Si gel (600 g) column using $CHCl_3$−MeOH−H_2O (80:20:1) as an eluent to afford fractions A−I. Fraction C (0.8 g) was subjected to preparative HPLC [Lop-ODS 5 × 100 cm; CH_3CN-H_2O (22:78) → (30:70) linear gradient] to afford **18** (96 mg), **19** (32 mg), **20** (39 mg), **21** (142 mg), and **22** (101 mg). Fraction E (6.4 g) was subjected to preparative HPLC [Lop-ODS 5 × 100 cm; CH_3CN-H_2O (22:78) → (30:70) linear gradient] to afford **2** (126 mg), **5** (23 mg), **8** (18 mg), **10** (21 mg), **11** (215 mg), **12** (186 mg) **13** (15 mg), **15** (82 mg), **16** (10 mg), and **23** (1.6 g). Fraction G (4.2 g) was subjected to preparative HPLC [Lop-ODS 5 × 100 cm; CH_3CN-H_2O (20:80) → (28:78) linear gradient] to afford **1** (88 mg), **3** (602 mg), **6** (127 mg), **7** (64 mg), **9** (70 mg), **14** (284 mg), and **17** (117 mg). Fraction H (2.46 g) was subjected to a preparative HPLC [Lop-ODS 5 × 100 cm; CH_3CN-H_2O (20:80) → (28:72) linear gradient] to afford **3** (165 mg) and **17** (22 mg).

Table 3. ^{1}H NMR Data of **11** and **12** in DMSO-d_6 at 35 °C

	11	**12**
aglycon moiety		
2	6.98 dd (8, 1)	6.98 d (8.5)
3	7.74 dd (8, 8)	7.74 dd (8.5, 8.5)
4	7.13 dd (8, 1)	7.13 dd (8.5, 1)
5	7.56 d (8)	7.55 d (8.5)
6	7.52 dd (8, 3)	7.51 dd (8.5, 3)
8	7.70 d (3)	7.67 d (3)
OMe	3.92 s	3.91 s
sugar moiety		
Glc-1	4.88 d (7.5)	4.92 d (7)
2	3.31 m	3.30 m
3	3.55 m	3.28 m
4	3.14 m	3.21 m
5	3.32 m	3.33 m
6	3.45 dd (11, 5)	3.52 dd (12, 2)
	3.91 m	3.72 dd (12, 5.5)
Api-1	4.84 d (2.5)	
2	3.86 dd (6, 2.5)	
4	3.92 d (9)	
	3.62 d (9)	
5	3.42 d (11)	
	3.44 d (11)	

Watterose A (1): amorphous powder, $[\alpha]^{23}_D$ −25.7° (*c* 0.98, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.45), 231 (4.50), 300 (4.50), 320 (4.59) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1157 $[M + Na]^+$.

Watterose B (2): amorphous powder, $[\alpha]^{23}_D$ −5.0° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.46), 231 (4.47), 301 (4.48), 319 (4.55) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1185 $[M + Na]^+$.

Watterose C (3): amorphous powder, $[\alpha]^{23}_D$ −6.6° (*c* 1.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.45), 230 (4.55), 300 (4.53), 321 (4.60) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1143 $[M + Na]^+$.

Watterose D (4): amorphous powder, $[\alpha]^{23}_D$ +17.5° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.57), 232 (4.61), 257 (4.31), 301 (4.51), 391 (4.60) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1101 $[M + Na]^+$.

Watterose E (5): amorphous powder, $[\alpha]^{23}_D$ −9.1° (*c* 1.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (sh) (4.52), 233 (sh) (4.49), 300 (4.41), 329 (4.57) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1215 $[M + Na]^+$.

Watterose F (6): amorphous powder, $[\alpha]^{23}_D$ −8.0° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.46), 233 (4.44), 300 (4.37), 329 (4.52) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1173 $[M + Na]^+$.

Watterose G (7): amorphous powder, $[\alpha]^{23}_D$ −12.1° (*c* 1.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.47), 233 (4.43), 300 (4.33), 331 (4.48) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1201$[M + H]^+$.

Watterose H (8): amorphous powder, $[\alpha]^{23}_D$ −41.2°(*c* 1.94, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (4.53), 283 (4.11), 301 (4.28), 314 (4.35) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1127 $[M + H]^+$.

Watterose I (9): amorphous powder, $[\alpha]^{23}_D$ −46.8° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.47), 231 (4.49), 284 (4.01), 301 (4.16), 328 (4.28) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1101 $[M + H]^+$.

Watterose J (10): amorphous powder, $[\alpha]^{27}_D$ −39.0° (*c* 1.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.57), 234 (4.52), 300 (4.44), 329 (4.55) nm; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1137 $[M + H]^+$.

Table 4. ^{13}C NMR Data of **11** and **12** in DMSO-d_6 at 35 °C

	11	**12**
aglycon moiety		
1	160.2	160.1
2	109.6	109.5
3	111.1	111.0
4	106.2	106.1
4a	157.4	157.3
4b	149.8	149.6
5	118.8	118.7
6	124.5	124.4
7	153.8	153.7
8	135.6	135.4
8a	122.8	122.8
8b	111.3	111.1
9	174.6	174.3
OMe	56.2	56.1
sugar moiety		
Glc-1	101.4	101.3
2	73.1	73.2
3	75.7	76.4
4	69.9	69.6
5	76.3	77.1
6	67.5	60.6
Api-1	109.4	
2	75.9	
3	78.7	
4	73.4	
5	63.3	

Wattersiixanthone A (11): amorphous powder, $[\alpha]^{27}_D$ −82.1° (*c* 1.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (4.34), 244 (4.36), 252 (4.41),281 (3.71), 304 (3.39), 362 (3.71) nm; ^1H and ^{13}C NMR, Tables 3 and 4; FABMS *m*/*z* 537 $[M + H]^+$, 559 $[M + Na]^+$.

Wattersiixanthone B (12): amorphous powder, $[\alpha]^{23}_D$ −20.5° (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (4.36), 234 (4.37), 252 (4.41), 281 (3.82), 305 (3.67), 357 (3.71) nm; ^1H and ^{13}C NMR, Tables 3 and 4; FABMS *m*/*z* 427 $[M + Na]^+$, 405 $[M+H]^+$.

Alkaline Hydrolysis of 1−10. Each compound (2 mg) was treated with 1 N aqueous NaOH (50 μL) for 4 h at room temperature in N_2 atmosphere, and the reaction mixture was extracted thrice with EtOAc after acidification with 1 N HCl, and the water layer was passed through a column equipped with Amberlite IR-120B and IRA-60E. From the H_2O eluate, tetrasaccharide (**13a**)[4] was detected from **1**−**10** by HPLC [Asahipak NH_2P-50, 4.6 mm × 25 cm, CH_3CN-H_2O (65:35), 1.0 mL/min, RI, t_R 6.3 min]. From the EtOAc layer, ferulic acid (8.5 min) was detected from **1**, **5**, and **6**, *p*-coumaric acid (7.6 min) was detected from **1**−**4** and **8**, caffeic acid (5.2 min) was detected from **2**−**7**, **9**, and **10**, benzoic acid (12.5 min) was detected from **1**−**10** by HPLC [YMC R-ODS-7, 4.6 mm × 25 cm, CH_3CN-H_2O (22.5:77.5) + 0.05% CF_3COOH, 1.0 mL/min, UV 270 nm].

The water eluate was concentrated under reduced pressure and was heated on a boiling water bath with 1 N HCl (50 μL) for 20 min. The reaction mixture was passed through an Amberlite IRA-60E column, and the eluate was concentrated. The residue was warmed at 60 °C with a solution of D-cysteine methyl ester in pyridine (3 mg/25 μL) for 90 min and to the reaction mixture hexamethyldisilazane (15 μL) and trimethylsilyl chloride (15 μL) were added, and the reaction mixture was stirred at 60 °C for 30 min. The supernatant was subjected to GC. Conditions: column Supelco SPB-1, 0.25 mm × 27 m; temperature 220 °C; carrier gas, N_2. D-Glucose (18.6 min) and D-fructose[8] were detected from **1**−**10**, and the ratio was 3:1.

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(8) Retention time for L-fructose (15.0 min) was obtained from its enantiomer (D-fructose + L-cysteine methyl ester).

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