

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/12344460>

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

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · SEPTEMBER 2000

Impact Factor: 3.8 · DOI: 10.1021/np000048q · Source: PubMed

CITATIONS

10

READS

11

5 AUTHORS, INCLUDING:



Hiroshi Noguchi

University of Shizuoka

199 PUBLICATIONS 3,370 CITATIONS

SEE PROFILE

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

Wataru Kobayashi,[†] Toshio Miyase,^{*,†} Sayako Suzuki,[†] Hiroshi Noguchi,[†] and Xin-Min Chen[‡]

School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan, and Division of Phytochemistry, Chengdu Institute of Biology, Academia Sinica, 4-9 Ren Ming Nan Lu, Chengdu, Sichuan, People's Republic of China

Received February 4, 2000

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*-feruloyl-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 wubangzicide 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 H₂O and extracted with ether. The H₂O 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 CHCl₃–MeOH–H₂O, 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 C₅₄H₆₂O₂₈. On acid hydrolysis, **1** gave D-glucose and D-fructose⁷ 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 ² J_{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 ¹³C NMR data were consistent with a molecular formula of C₅₃H₆₂O₂₉. The ¹H and ¹³C 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 ¹³C NMR data were consistent with a molecular formula of C₅₁H₆₀O₂₈. The ¹H and ¹³C 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**.

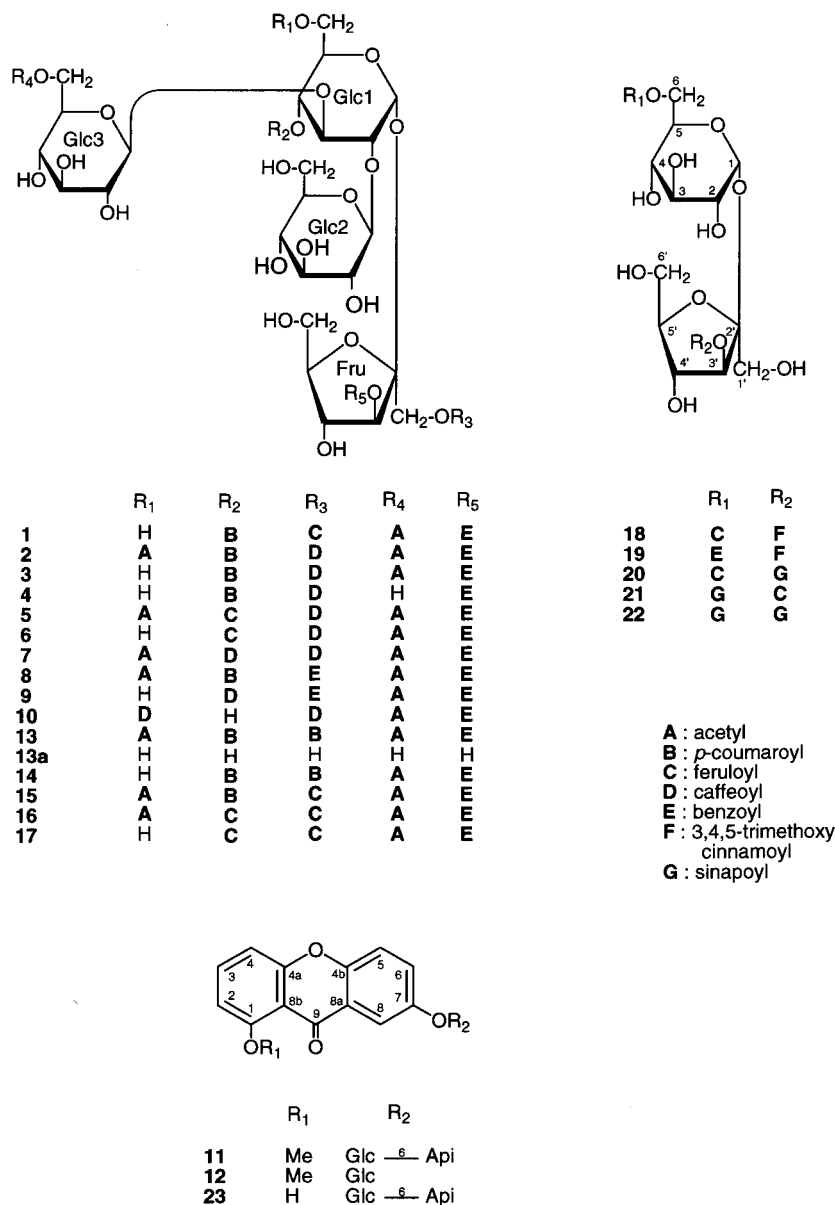
The FABMS of watterose D (**4**) gave a quasimolecular ion peak at m/z 1101 [M + Na]⁺, 42 mass units lower

* To whom correspondence should be addressed. Tel.: +81 054-264-5661. Fax: +81 054-264-5661. E-mail: miyase@ys7.u-shizuoka-ken.ac.jp.

[†] University of Shizuoka.

[‡] Academia Sinica.

Chart 1



than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{49}\text{H}_{58}\text{O}_{27}$. 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 $[\text{M} + \text{Na}]^+$, 30 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{54}\text{H}_{64}\text{O}_{30}$. 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 $[\text{M} + \text{Na}]^+$, 30 mass units higher than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{53}\text{H}_{62}\text{O}_{29}$. 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 $[\text{M} + \text{Na}]^+$, 16 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{53}\text{H}_{62}\text{O}_{30}$. 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 $[\text{M} + \text{Na}]^+$, 58 mass units higher than that of **2**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{51}\text{H}_{60}\text{O}_{25}$. 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. ^1H NMR Data of **1–10** in CD_3OD at 35°C^a

Sugar moiety	1	2	3	4	5	6	7	8	9	10
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)	5.00 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)
Glc2-1	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
2	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)
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
Glc3-1	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
2	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)
3	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
4	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
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	3.24 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)	3.22 m
Fru-1	4.22 d (12)	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
2	4.73 d (12)	4.71 d (12)	4.74 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)
3	5.72 d (8)	5.72 d (8)	5.72 d (8)	5.72 d (8)	5.72 d (8)	4.76 d (12)	4.71 d (12)	4.85 d (12)	4.86 d (12)	4.71 d (12)
4	4.51 dd (8, 8)	5.72 d (8)	4.53 dd (8, 8)	4.52 dd (8, 8)	4.43 dd (8.5, 8.5)	5.74 d (8)	5.72 d (8)	5.79 d (8)	5.78 d (8)	5.73 d (8)
5	4.04 ^b	4.07 ^b	4.04 ^b	4.03 ^b	4.07 ^b	4.07m	4.44 dd (8, 8)	4.46 t (8)	4.53 dd (8, 8)	4.50 dd (8, 8)
6	3.87 ^b	3.86 ^b	3.87 ^b	3.87 ^b	3.86 ^b	3.90 ^b	4.06 ^b	4.08 m	4.04 m	4.06 ^b
Ac (R ₁)	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
Ac (R ₂)	1.63 s	2.06 s	1.63 s	3.87 ^b	2.06 s	2.06 s	2.06 s	2.07 s	3.86 ^b	3.83 dd (12, 2.5)
acid	1.63 s	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
(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)	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)	7.58 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)	6.88 d (8)	6.82 d (8)	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)	6.85 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)	6.85 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	6.31 d (16)	6.31 d (16)	8.10 dd (7.5, 1.5)	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)	7.62 d (16)	7.62 d (16)	7.61 tt (7.5, 1.5)	7.47 t (7.5)
2	7.20 d (2)	7.05 d (2)	7.06 d (2)	7.05 d (2)	7.82 d (16)	7.66 d (16)	7.05 d (2)	8.10 dd (7.5, 1.5)	7.46 t (7.5)	6.77 d (8)
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)	7.46 t (7.5)	8.10 dd (7.5, 1.5)	6.89 dd (8, 2)
6	7.03 dd (8, 2)	6.91 dd (8, 2)	6.92 dd (8, 2)	6.93 dd (8.5, 2)	7.82 d (8)	6.82 d (8)	6.76 d (8)	7.61 tt (7.5, 1.5)	8.18 dd (7.5, 1.5)	8.15 dd (7, 1)
OMe	3.91 s	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)
(at C-3 of Fru) α	8.20 br d (7.5)	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)
3	7.59 t (8)	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)	7.72 tt (7.5, 1.5)	7.54 t (7)
4	7.70 tt (7.1)	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)	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)	8.18 dd (7.5, 1.5)	8.15 dd (7, 1)

^a Assigned with the aid of HOHAHA difference, ^1H – ^1H 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 $[\text{M} + \text{Na}]^+$, 42 mass units higher than that of **3**, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{47}\text{H}_{58}\text{O}_{27}$. 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 $[\text{M} + \text{H}]^+$, and ^{13}C NMR data were consistent with a molecular formula of $\text{C}_{51}\text{H}_{60}\text{O}_{29}$. 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, $^nJ_{\text{C-H}}$ 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 H₂-6 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 H₂-1 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 wubangzisi 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. 1H (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 \times 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 \times 100 cm; CH_3CN - H_2O (22:78) \rightarrow (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 \times 100 cm; CH_3CN - H_2O (22:78) \rightarrow (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 \times 100 cm; CH_3CN - H_2O

Table 3. 1H NMR Data of **11** and **12** in DMSO- d_6 at 35 $^\circ C$

	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)	

(20:80) \rightarrow (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 \times 100 cm; CH_3CN - H_2O (20:80) \rightarrow (28:72) linear gradient] to afford **3** (165 mg) and **17** (22 mg).

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

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

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

Watterose D (4): amorphous powder, $[\alpha]^{23}_D +17.5^\circ$ (*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; 1H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1101 $[M + Na]^+$.

Watterose E (5): amorphous powder, $[\alpha]^{23}_D -9.1^\circ$ (*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; 1H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1215 $[M + Na]^+$.

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

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

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

Watterose I (9): amorphous powder, $[\alpha]^{23}_D -46.8^\circ$ (*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; 1H and ^{13}C NMR, Tables 1 and 2; FABMS m/z 1101 $[M + H]^+$.

Watterose J (10): amorphous powder, $[\alpha]^{27}_D -39.0^\circ$ (*c* 1.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.57), 234 (4.52), 300 (4.44), 329 (4.55) nm; 1H 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 $\text{DMSO}-d_6$ 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, $[\alpha]_{\text{D}}^{27} -82.1^\circ$ (*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 $[\text{M} + \text{H}]^+$, 559 $[\text{M} + \text{Na}]^+$.

Wattersiixanthone B (12): amorphous powder, $[\alpha]_{\text{D}}^{23} -20.5^\circ$ (*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 $[\text{M} + \text{Na}]^+$, 405 $[\text{M} + \text{H}]^+$.

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 NH₂P-50, 4.6 mm \times 25 cm, $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (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 \times 25 cm, $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (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 \times 27 m; temperature 220 °C; carrier gas, N_2 . D-Glucose (18.6 min) and D-fructose⁸ were detected from **1–10**, and the ratio was 3:1.

Acknowledgment. We thank the staff of the Analytical Center, University of Shizuoka, for measurement of FABMS.

References and Notes

- (1) Zhang, D.-M.; Miyase, T.; Kuroyanagi, M.; Umehara, K.; Noguchi, H. *Phytochemistry* **1998**, *47*, 45–52, and references cited therein.
- (2) Saitoh, H.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull.* **1994**, *42*, 1879–1885.
- (3) Zhang, D.-M.; Miyase, T.; Kuroyanagi, M.; Umehara, K.; Noguchi, H. *Phytochemistry* **1997**, *45*, 773–741, and references cited therein.
- (4) Saitoh, H.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull.* **1993**, *41*, 2125–2128.
- (5) Miyase, T.; Ueno, A. *Shouyugaku Zasshi* **1993**, *47*, 267–278.
- (6) Pan, M.; Mao, Q. *Yaoxue Xueao* **1984**, *19*, 899–903.
- (7) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843–1845.
- (8) Retention time for L-fructose (15.0 min) was obtained from its enantiomer (D-fructose + L-cysteine methyl ester).

NP000048Q