Cucurbitosides F−M, Acylated Phenolic Glycosides from the Seeds of *Cucurbita pepo*

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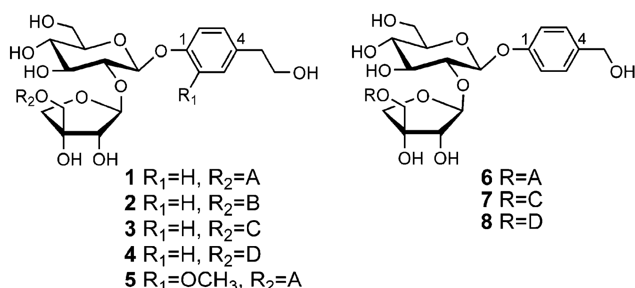
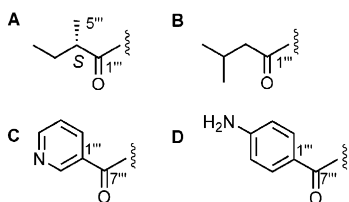
Eight new phenolic glycosides, cucurbitosides F−M (**1**−**8**), were isolated from the seeds of *Cucurbita pepo*. Their structures were elucidated as 4-(2-hydroxyethyl)phenyl 5-*O*-(2-*S*-2-methylbutyryl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**1**), 4-(2-hydroxyethyl)phenyl 5-*O*-(3-methylbutyryl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**2**), 4-(2-hydroxyethyl)phenyl 5-*O*-nicotinyl-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**3**), 4-(2-hydroxyethyl)phenyl 5-*O*-(4-aminobenzoyl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**4**), 4-(2-hydroxyethyl)-2-methoxyphenyl 5-*O*-(2-*S*-2-methylbutyryl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**5**), 4-(hydroxymethyl)phenyl 5-*O*-(2-*S*-2-methylbutyryl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**6**), 4-(hydroxymethyl)phenyl 5-*O*-nicotinyl-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**7**), and 4-(hydroxymethyl)phenyl 5-*O*-(4-aminobenzoyl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside (**8**) on the basis of various spectroscopic analyses and analyses of hydrolysis products.

The fruits of *Cucurbita* species, common name pumpkin, are well-known vegetables throughout the world, and the seeds are eaten as snacks after salting or roasting. In China, the seeds of *C. moschata* have been traditionally used as deworming medicine.[1] In Europe, the seeds of *C. pepo* have been used medicinally in the treatment of urinary and prostate disease.[2] In a previous study, we reported five acylated phenolic glycosides, cucurbitosides A−E, from the seeds of *C. moschata*.[3] As our current interest involves the chemistry of biologically active natural products, we investigated the chemical constituents of the seeds of *C. pepo*, which resulted in the isolation of eight additional acylated phenolic glycosides, cucurbitosides M−F (**1**−**8**). This paper deals with the isolation and structure elucidation of these new compounds.





Results and Discussion

The seeds of *C. pepo* were extracted with MeOH. The methanolic extract was partitioned between *n*-hexane and H_2O. The H_2O layer was passed through a Diaion HP-20 column and washed with MeOH. The methanolic eluate was evaporated and fractionated using an ODS column. Further purification by repeated reversed-phase HPLC afforded the new phenolic glycosides, which were termed cucurbitosides F−M (**1**−**8**).

Cucurbitoside F (**1**) was isolated as an amorphous solid. The molecular formula was established as $C_{24}H_{36}O_{12}$ by HRFABMS. On acid hydroysis, **1** afforded D-glucose and D-apiose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives. The ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) revealed signals assignable to *β*-glucopyranosyl and *β*-apiofuranosyl moieties. Further, in the ^{1}H NMR spectrum, the A_2B_2-type aromatic proton signals at δ 6.97 (2H, d, J = 8.7 Hz) and 7.13 (2H, d, J = 8.7 Hz) and the signals due to a benzylic methylene at δ 2.76 (2H, t, J = 7.1 Hz) and a hydroxymethyl at δ 3.70 (2H, t, J = 7.1 Hz) suggested the presence of the 4-(2-hydroxyethyl)phenol moiety in **1**, which was confirmed by the HMBC correlation between δ_H 2.76 (H_2-7) and δ_C 131.0 (C-3 and C-5). The presence of the 2-methylbutyryl moiety in **1** was suggested from the proton signals of a primary methyl at δ 0.82 (3H, t, J = 7.5 Hz), a secondary methyl at δ 1.03 (3H, d, J = 6.9 Hz), a methylene at δ 1.38, 1.56 (each 1H, m), and a methine at δ 2.28 (1H, m), and the carbonyl carbon signal at δ_C 178.0. Comparison of the ^{1}H and ^{13}C NMR spectra of **1** and cucurbitoside A[3] revealed that the signals assignable to the aglycon and sugar moieties were superimposable, but differences were observed in the acyl moiety, suggesting the same 4-(2-hydroxyethyl)phenyl *β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside moiety in **1** as in cucurbitoside A. The connections of the aglycon, component sugars, and the acyl moiety were confirmed from the HMBC correlations between δ_H 4.93 (Glc-H-1′) and δ_C 157.4 (C-1), δ_H 5.46 (Api-H-1″) and δ_C 78.7 (Glc-C-2′), and δ_H 4.12, 4.09 (Api-H-5″) and δ_C 178.0 (C-1‴). To determine the absolute stereochemistry of the 2-methylbutyryl moiety, alkaline hydrolysis of **1** was carried out to afford 2-methylbutyric acid (**9**), which was subsequently esterificated with (*R*)-pantolactone (**10**) to give the ester (**11**) (Scheme 1). Comparison of the ^{1}H NMR data of **11** with the synthetic 2-*S*-2-methylbutyryl-*R*-pantolactone and racemic 2-*RS*-2-methylbutyryl-*R*-pan-

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Table 1. ^{1}H NMR Data (500 MHz) for **1**−**8** in Methanol-d_4

position	1	2	3	4	6	7	8	position	5
2 and 6	6.97 (d, 8.7)	6.97 (d, 8.7)	6.86 (d, 8.5)	6.90 (d, 8.7)	7.02 (d, 8.7)	6.92 (d, 8.5)	7.12 (d, 8.5)	3	6.84 (d, 2.1)
3 and 5	7.13 (d, 8.7)	7.13 (d, 8.7)	6.94 (d, 8.5)	6.96 (d, 8.7)	7.26 (d, 8.7)	7.08 (d, 8.5)	6.95 (d, 8.5)	6	7.00 (d, 8.3)
								5	6.73 (dd, 8.3, 2.1)
7	2.76 (t, 7.1)	2.76 (t, 7.1)	2.63 (m)	2.67 (t, 7.1)	4.52 (s)	4.41 (s)	4.45 (s)	7	2.76 (t, 7.1)
8	3.70 (t, 7.1)	3.70 (t, 7.1)	3.61 (t, 7.1)	3.63 (t, 7.1)				8	3.72 (t, 7.1)
								OCH_3	3.82 (s)
Glc-1′	4.93 (d, 7.5)	4.93 (d, 7.6)	4.94 (d, 7.6)	4.90 (d, 7.8)	4.96 (d, 7.5)	4.97 (d, 7.5)	4.94 (d, 7.5)		4.95 (d, 7.7)
2′	3.64 (dd, 9.2, 7.5)	3.64 (dd, 9.2, 7.6)	3.68 (dd, 9.1, 7.6)	3.65 (dd, 9.1, 7.8)	3.65 (dd, 9.2, 7.5)	3.69[a]	3.67 (dd, 8.9, 7.5)		3.70 (dd, 9.2, 7.7)
3′	3.59 (t, 9.2)	3.58 (t, 9.2)	3.61[a]	3.59 (t, 9.1)	3.59 (t, 9.2)	3.62 (t, 9.0)	3.60 (t, 8.9)		3.59 (t, 9.2)
4′	3.40[a]	3.38[a]	3.39[a]	3.37[a]	3.39 (t, 9.2)	3.37 (t, 9.0)	3.38 (t, 8.9)		3.39 (dd, 9.2, 8.5)
5′	3.40 (m)	3.38 (m)	3.39 (m)	3.37 (m)	3.41 (m)	3.42 (m)	3.41 (m)		3.35 (m)
6′	3.67 (dd, 11.7, 5.5)	3.67[a]	3.67 (dd, 12.1, 5.0)	3.67 (dd, 12.2, 5.1)	3.68 (dd, 12.0, 5.3)	3.69[a]	3.68 (dd, 12.1, 5.3)		3.66 (dd, 12.1, 5.3)
	3.87 (dd, 11.7, 1.1)	3.86 (dd, 12.4, 1.8)	3.86 (dd, 12.1, 1.8)	3.86 (dd, 12.2, 1.9)	3.87 (dd, 12.0, 1.9)	3.86 (dd, 12.1, 2.0)	3.86 (dd, 12.1, 1.9)		3.83 (dd, 12.1, 2.0)
Api-1″	5.46 (d, 1.4)	5.46 (d, 1.4)	5.52 (d, 0.8)	5.50 (d, 1.2)	5.46 (d, 1.6)	5.51 (s)	5.49 (d, 0.7)		5.52 (d, 1.4)
2″	3.91 (d, 1.4)	3.89 (d, 1.4)	4.02 (br.s)	3.97 (d, 1.2)	3.91 (d, 1.6)	4.03 (s)	3.99 (br.s)		3.93 (d, 1.4)
4″	3.82 (d, 9.8)	3.81 (d, 9.9)	3.91 (d, 9.6)	3.89 (d, 9.8)	3.82 (d, 9.7)	3.91 (d, 9.9)	3.89 (d, 9.8)		3.77 (d, 9.8)
	4.13 (d, 9.8)	4.14 (d, 9.9)	4.31 (d, 9.6)	4.31 (d, 9.8)	4.12 (d, 9.7)	4.30 (d, 9.9)	4.29 (d, 9.8)		4.26 (d, 9.8)
5″	4.09 (d, 11.5)	4.08 (d, 11.4)	4.32 (d, 11.5)	4.18 (d, 11.2)	4.08 (d, 11.4)	4.33 (d, 11.4)	4.19 (d, 11.4)		4.12 (s)
	4.12 (d, 11.5)	4.11 (d, 11.4)	4.42 (d, 11.5)	4.26 (d, 11.2)	4.11 (d, 11.4)	4.42 (d, 11.4)	4.26 (d, 11.4)		
2‴	2.28 (m)	2.08 (d, 7.6)	9.01 (d, 1.6)	7.66 (d, 8.9)	2.28 (m)	9.00 (d, 1.8)	7.65 (d, 8.7)		2.26 (m)
3‴	1.38 (m)	1.38 (m)			1.38 (m)				1.35 (m)
	1.56 (m)			6.58 (d, 8.9)	1.57 (m)		6.56 (d, 8.7)		1.54 (m)
4‴	0.82 (t, 7.5)	0.87 (d, 6.4)[b]	8.71 (dd, 5.8, 1.6)		0.82 (t, 7.4)	8.70 (dd, 5.0, 1.8)			0.80 (t, 7.5)
5‴	1.03 (d, 6.9)	0.88 (d, 6.4)[b]	7.48 (dd 8.0, 5.1)	6.58 (d, 8.9)	1.03 (d, 6.9)	7.48 (dd 8.0, 5.0)	6.56 (d, 8.7)		1.01 (d, 7.1)
6‴			8.25 (dt, 8.0, 1.6)	7.66 (d, 8.9)		8.25 (dt, 8.0, 1.8)	7.65 (d, 8.7)		

[a] Overlapped signals. [b] Assignments may be interchanged.

tolactone revealed the absolute configuration of the 2-methylbutyryl moiety in **1** to be 2-*S*. Thus, cucurbitoside F (**1**) was established as 4-(2-hydroxyethyl)phenyl 5-*O*-(2-*S*-2-methylbutyryl)-β-D-apiofuranosyl(l→2)-β-D-glucopyranoside.

Cucurbitoside G (**2**) was isolated as an amorphous solid. The HRFABMS data determined the same molecular formula, $C_{24}H_{36}O_{12}$, as that of **1**. The ^{1}H and ^{13}C NMR data of **2** and **1** were superimposable, except for the replacement of the signals of the primary methyl in the acyl moiety in **1** by those of a secondary methyl in **2**. Further analysis of the 2D-NMR data, including DQFCOSY, HMQC, and HMBC spectra, suggested the acyl moiety to be the 3-methylbutyryl moiety. Thus, cucurbitoside G (**2**) was established as 4-(2-hydroxyethyl)phenyl 5-*O*-(3-methylbutyryl)-β-D-apiofuranosyl(l→2)-β-D-glucopyranoside.

Cucurbitoside H (**3**) was isolated as an amorphous solid. The molecular formula was established as $C_{25}H_{31}O_{12}N$ by HRFABMS. The 4-(2-hydroxyethyl)phenyl β-D-apiofuranosyl(l→2)-β-D-glucopyranoside moiety in **3** was the same as **1** and **2**, as suggested from the ^{1}H and ^{13}C NMR data. The proton and carbon signals assignable to the acyl moiety were observed as four aromatic protons at δ_H 7.48 (1H, dd, J = 8.0, 5.1 Hz), 8.25 (1H, dt, J = 8.0, 1.6 Hz), 8.71 (1H, dd, J = 5.8, 1.6 Hz), and 9.01 (1H, d, J = 1.6 Hz), with the corresponding carbon signals at δ_C 125.1, 138.9, 154.0, and 151.2, a carbonyl carbon signal at δ_C 165.9, and an aromatic carbon signal at δ_C 127.6. Taking the molecular formula into consideration, it was apparent that the acyl moiety was nicotinyl. Thus, cucurbitoside H (**3**) was established as 4-(2-hydroxyethyl)phenyl 5-*O*-nicotinyl-β-D-apiofuranosyl(l→2)-β-D-glucopyranoside.

Cucurbitoside I (**4**) was isolated as an amorphous solid. The molecular formula was established as $C_{26}H_{33}O_{12}N$ by HRFABMS. When the ^{1}H and ^{13}C NMR data of **4** were compared with those of cucurbitoside B,[3] it revealed superimposable signals, except that some shifts were observed at C-4‴ (−8.7 ppm), C-3‴,5‴ (+1.8 ppm), C-1‴ (+2.2 ppm), H-3‴,5‴ (−0.17 ppm), and H-2‴,6‴ (−0.13 ppm). Taking the molecular formula into consideration, it was suggested that the hydroxyl at C-4‴ of the acyl moiety in cucurbitoside B was replaced by an amino moiety in **4**. Thus, cucurbitoside I (**4**) was established as 4-(2-hydroxyethyl)phenyl 5-*O*-(4-aminobenzoyl)-β-D-apiofuranosyl(l→2)-β-D-glucopyranoside.

Cucurbitoside J (**5**) was isolated as an amorphous solid. The molecular formula was established as $C_{25}H_{38}O_{13}$ by HRFABMS. The ^{1}H NMR spectrum of **5**, in addition to the superimposable signals due to the β-D-apiofuranosyl(l→2)-β-D-glucopyranose and the 2-methylbutyryl moieties as those of **1**, also showed a set of ABC-type trisubstituted aromatic proton signals at δ 6.73 (1H, dd, J = 8.3, 2.1 Hz), 6.84 (1H, d, J = 2.1 Hz), and 7.00 (d, J = 8.3 Hz), the signals of a benzylic methylene at δ 2.76 (2H, t, J = 7.1 Hz) and a hydroxymethyl at δ 3.72 (2H, t, J = 7.1 Hz), and a methoxy singlet at δ 3.82, suggesting the presence of the 4-(2-hydroxyethyl)-2-methoxyphenol moiety in **5**, of which the position of the methoxy group was determined at C-2 by the NOESY correlation between δ_H 3.82 (OCH_3) and δ_H 6.84 (H-3). The absolute stereochemistry of the 2-methylbutyryl moiety was determined to be 2-*S* by the same method as carried out on **1**. On the basis of the above evidence, cucurbitoside J (**5**) was established as 4-(2-hydroxyethyl)-2-methoxyphenyl 5-*O*-(2-*S*-2-methylbutyryl)-β-D-apiofuranosyl(l→2)-β-D-glucopyranoside.

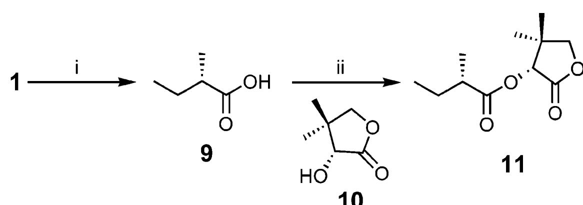
Cucurbitoside K (**6**) was isolated as an amorphous solid. The molecular formula was established as $C_{23}H_{34}O_{12}$ by

Table 2. ^{13}C NMR (125 MHz) Data for **1**−**8** in Methanol-d_4

position	**1**	**2**	**3**	**4**	**6**	**7**	**8**	position	**5**
1	157.4	157.5	157.1	157.4	158.3	158.0	158.2	1	150.9
2 and 6	117.6	117.5	117.1	117.4	117.4	117.1	117.3	2	146.3
3 and 5	131.0	131.0	130.8	130.9	129.5	129.4	129.5	3	122.3
4	134.3	134.2	134.0	134.1	136.7	136.5	136.5	4	135.1
								5	114.6
								6	117.7
7	39.5	39.5	39.3	39.4	64.8	64.8	64.9	7	39.9
8	64.4	64.4	64.3	64.3				8	64.4
								OCH_3	56.5
Glc-1′	101.0	100.9	100.5	101.0	100.8	100.5	100.9		101.0
2′	78.7	78.4	78.1	78.4	78.7	78.2	78.5		78.1
3′	78.8	78.8	78.8	78.8	78.8	78.9	78.8		78.7
4′	71.6	71.6	71.6	71.6	71.6	71.6	71.6		71.6
5′	78.0	78.1	78.0	78.1	78.1	78.1	78.1		77.6
6′	62.6	62.6	62.5	62.6	62.6	62.6	62.6		62.6
Api-1″	110.6	110.5	110.4	110.5	110.6	110.5	110.6		110.1
2″	78.8	78.9	78.6	78.9	78.8	78.7	78.9		79.0
3″	79.1	79.1	79.1	79.3	79.1	79.2	79.3		79.2
4″	75.4	75.4	75.3	75.5	75.4	75.4	75.5		75.5
5″	67.5	67.7	68.7	67.8	67.5	68.8	67.6		67.7
1‴	178.0	174.5	127.6	118.4	178.0	127.7	118.4		178.1
2‴	42.2	44.0	151.2	132.8	42.2	151.3	132.8		42.2
3‴	27.7	26.8		114.4	27.7		114.4		27.7
4‴	11.9	22.8[a]	154.0	154.9	11.9	154.1	154.9		11.9
5‴	16.8	22.7[a]	125.1	114.4	16.8	125.2	114.4		16.8
6‴			138.9	132.8		139.0	132.8		
7‴			165.9	168.4		165.9	168.4		

[a] Assignments may be interchanged.

Scheme 1[a]



[a] (i) 5% KOH, 40 °C, 1 h. (ii) BF_3 etherate, 80 °C, 1 h.

HRFABMS. The ^{1}H and ^{13}C NMR data suggested that **6** differed from **1** by the replacement of the 2-hydroxyethyl moiety in the aglycon of **1** with the hydroxymethyl moiety. Namely, the signals of the hydroxymethyl moiety were observed at δ_H 4.52 (2H, s) and δ_C 64.8. Further comparison of the ^{1}H and ^{13}C NMR data of **6** and cucurbitoside C[3] showed superimposable signals assignable to the 4-(hydroxymethyl)phenol moiety and the sugar chain. The structure of **6** was confirmed by detailed analysis of the 2D-NMR data, including DQF-COSY, HMQC, and HMBC spectra. Thus, cucurbitoside K (**6**) was elucidated as 4-(hydroxymethyl)phenyl 5-*O*-(2-*S*-2-methylbutyryl)-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside.

Cucurbitoside L (**7**) was isolated as an amorphous solid. The molecular formula was established as $C_{24}H_{29}O_{12}N$ by HRFABMS. Comparison of the ^{1}H and ^{13}C NMR data of **7** with those of **3** and **6** suggested the presence of the 4-(hydroxymethyl)phenol moiety as the aglycon, *β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranose as the sugar chain, and nicotinyl as the acyl moiety. The connection of each group was confirmed by the HMBC data. Thus, the structure of cucurbitoside L (**7**) was established as 4-(hydroxymethyl)phenyl 5-*O*-nicotinyl-*β*-D-apiofuranosyl-(l→2)-*β*-D-glucopyranoside.

Cucurbitoside M (**8**) was isolated as an amorphous solid. The molecular formula was established as $C_{25}H_{31}O_{12}N$ by HRFABMS. Comparison of the ^{1}H and ^{13}C NMR data of **7** with those of **4** and **6** suggested the presence of the 4-(hydroxymethyl)phenol moiety as the aglycon and *β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranoside as the sugar chain, and the acyl moiety was assigned to be 4-aminobenzoyl. Thus, cucurbitoside M (**8**) was established as 4-(hydroxymethyl)phenyl 5-*O*-(4-aminobenzoyl)-*β*-D-apiofuranosyl-(l→2)-*β*-D-glucopyranoside.

In conclusion, we have isolated eight new acylated phenolic glycosides, cucurbitosides F−M (**1**−**8**), from the seeds of *C. pepo*. The structures of **1**−**8** are closely related to the previously isolated cucurbitosides A−E from the seeds of *C. moschata*.[3] All these compounds contain the 5-*O*-acyl-*β*-D-apiofuranosyl(l→2)-*β*-D-glucopyranose moiety in the structures, which appears to be a characteristic constituent of pumpkin seeds.

Experimental Section

General Experimental Procedures. The UV spectra were obtained with a Shimadzu Biospec-Mini spectrophotometer, and the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm cell. The ^{1}H and ^{13}C NMR measurements were recorded using a JEOL ECP-500 NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). FABMS and HRFABMS were conducted using a JEOL JMS-700 MStation mass spectrometer. Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan) and ODS (Chromatorex, 100−200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography. For HPLC, a JASCO PU-1580 HPLC system, equipped with a Shodex RI-71 differential refractometer detector, was used. TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS instrument.

Plant Material. The seeds of *C. pepo* were purchased from a seeds shop in Chiba, Japan, in November 2003, and identified by one of authors (T.N.). A voucher specimen was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Toho University, Japan.

Extraction and Isolation. The seeds (5.0 kg) were extracted with MeOH with ultrasonic treatment three times for 1 h each at room temperature. The methanolic extract was concentrated (87 g), suspended in H_2O, and then partitioned

successively with *n*-hexane. The H_2O layer was passed through a Diaion HP-20 column and washed with H_2O and MeOH. The MeOH fraction (12.4 g) was chromatographed over an ODS column eluted with 40% and 80% MeOH to give two fractions, A (4.2 g) and B (2.8 g). Further purification of fraction A by repeated preparative HPLC with 40% MeOH and 20% CH_3-CN afforded eight compounds, **1** (166 mg), **2** (5 mg), **3** (9 mg), **4** (7 mg), **5** (4 mg), **6** (51 mg), **7** (4 mg), and **8** (6 mg).

Cucurbitoside F (1): amorphous solid, $[\alpha]_D^{22}$ −89.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 272 (3.06), 221 (3.96); IR (KBr) ν_{max} 3366, 1726, 1610, 1510, 1459, 1383, 1234 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 515 $[M - H]^-$; HRFABMS (negative) *m/z* 515.2143 $[M - H]^-$ (calcd for $C_{24}H_{35}O_{12}$, 515.2129).

Cucurbitoside G (2): amorphous solid, $[\alpha]_D^{22}$ −81.3 (*c* 0.25, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 272 (3.14), 221 (4.00); IR (KBr) ν_{max} 3406, 1730, 1615, 1510, 1459, 1381, 1234 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (positive) *m/z* 539 $[M + Na]^+$; HRFABMS (positive) *m/z* 539.2104 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_{12}Na$, 539.2104).

Cucurbitoside H (3): amorphous solid, $[\alpha]_D^{22}$ −78.5 (*c* 0.80, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 264 (3.50), 219 (4.21); IR (KBr) ν_{max} 3393, 1724, 1612, 1511, 1381, 1287, 1233 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 536 $[M - H]^-$; HRFABMS (negative) *m/z* 536.1786 $[M - H]^-$ (calcd for $C_{25}H_{30}O_{12}N$, 536.1768).

Cucurbitoside I (4): amorphous solid, $[\alpha]_D^{22}$ −57.1 (*c* 0.44, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 295 (4.25), 220 (4.19); IR (KBr) ν_{max} 3378, 1691, 1604, 1512, 1380, 1277 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 550 $[M - H]^-$; HRFABMS (negative) *m/z* 550.1951 $[M - H]^-$ (calcd for $C_{26}H_{32}O_{12}N$, 550.1925).

Cucurbitoside J (5): amorphous solid, $[\alpha]_D^{22}$ −58.4 (*c* 0.18, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 277 (3.31), 222 (3.81); IR (KBr) ν_{max} 3404, 1729, 1597, 1513, 1460, 1382, 1266 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 545 $[M - H]^-$; HRFABMS (negative) *m/z* 545.2255 $[M - H]^-$ (calcd for $C_{25}H_{37}O_{13}$, 545.2235).

Cucurbitoside K (6): amorphous solid, $[\alpha]_D^{22}$ −87.7 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 271 (3.12), 221 (3.94); IR (KBr) ν_{max} 3393, 1724, 1612, 1511, 1459, 1383, 1234 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 501 $[M - H]^-$; HRFABMS (negative) *m/z* 501.1972 $[M - H]^-$ (calcd for $C_{23}H_{33}O_{12}$, 501.1972).

Cucurbitoside L (7): amorphous solid, $[\alpha]_D^{22}$ −80.6 (*c* 0.39, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 264 (3.50), 220 (4.19); IR (KBr) ν_{max} 3406, 1723, 1613, 1512, 1382, 1293, 1234 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 522 $[M - H]^-$; HRFABMS (negative) *m/z* 522.1629 $[M - H]^-$ (calcd for $C_{24}H_{28}O_{12}N$, 522.1611).

Cucurbitoside M (8): amorphous solid, $[\alpha]_D^{22}$ −69.7 (*c* 0.30, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 294 (4.26), 221 (4.21); IR (KBr) ν_{max} 3379, 1690, 1605, 1513, 1376, 1278 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Tables 1 and 2; FABMS (negative) *m/z* 536 $[M - H]^-$; HRFABMS (negative) *m/z* 536.1792 $[M - H]^-$ (calcd for $C_{25}H_{30}O_{12}N$, 536.1768).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides. Each solution of **1**−**8** (each 0.5 mg), in 1 M HCl (dioxane−H_2O, 1:1, 200 μL), was heated at 100 °C for 1 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY-1 (30 m × 0.25 mm × 0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier, N_2 gas. In the acid hydrolysate of **1**−**8**, D-glucose and D-apiose were confirmed by comparison of the retention times of their derivatives with those of D-glucose, L-glucose, and D-apiose derivatives prepared in a similar way, which showed retention times of 11.00, 11.45, and 6.09 min, respectively.

Alkaline Hydrolysis and Determination of the Absolute Configuration of the 2-Methylbutyryl Moieties in 1, 5, and 6. Compound **1** (3 mg) and the mixture of **1**, **5**, and **6** (each 1 mg) were treated with 5% KOH (200 μL) for 1 h at 40 °C, respectively. The solution was neutralized with 1 M HCl and partitioned with $CHCl_3$ (300 μL). The $CHCl_3$ layer was dried with Na_2SO_4 and filtered through a filter paper to remove the Na_2SO_4. (*R*)-Pantolactone (3 mg) and 2 drops of BF_3 etherate were added to the filtrate, and the mixture was heated at 80 °C for 1 h. H_2O (100 μL) was added to the reaction solution, then the mixture was extracted with *n*-hexane (1 mL). The *n*-hexane layer was dried with Na_2SO_4, filtered, and evaporated to afford the ester **11**. 2-*S*-2-Methylbutyryl-*R*-pantolactone and racemic 2-*RS*-2-methylbutyryl-*R*-pantolactone were synthesized from commercial 2-*S*-2-methylbutyric acid and 2-*RS*-2-methylbutyric acid with (*R*)-pantolactone by the same method mentioned above. The ^1H NMR spectra of **11**, 2-*S*-2-methylbutyryl-*R*-pantolactone, and racemic 2-*RS*-2-methylbutyryl-*R*-pantolactone were recorded in $CDCl_3$ with TMS as the internal reference at 30 °C, and the ^1H NMR spectrum of **11** was identical with that of 2-*S*-2-methylbutyryl-*R*-pantolactone.[4] Characteristic signals were δ 5.38 (1H, s) and 0.96 (3H, t, $J = 7.6$ Hz), in contrast to the ^1H NMR spectrum of racemic 2-*RS*-2-methylbutyryl-*R*-pantolactone, which showed characteristic signals of 2-*R*-2-methylbutyryl-*R*-pantolactone at δ 5.37 (1H, s) and 0.97 (3H, t, $J = 7.6$ Hz).

References and Notes

(1) State Administration of Traditional Chinese Medicine. *Zhong-hua-ben-cao*; Shanghai Science and Technology Press: Shanghai, 1999; p 4600.

(2) Bombardelli, E.; Morazoni, P. *Fitoterapia* **1997**, *68*, 291−302.

(3) Koike, K.; Li, W.; Liu, L.; Hata, E.; Nikaido, T. *Chem. Pharm. Bull.* **2005**, *53*, 225−228.

(4) Barbeni, M.; Allegrone, G.; Cisero, M.; Guarda, P. A. *Flavour Frag. J.* **1992**, *7*, 163−167.

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