Eight New Cucurbitane Glycosides, Kuguaglycosides A – H, from the Root of *Momordica charantia* L.

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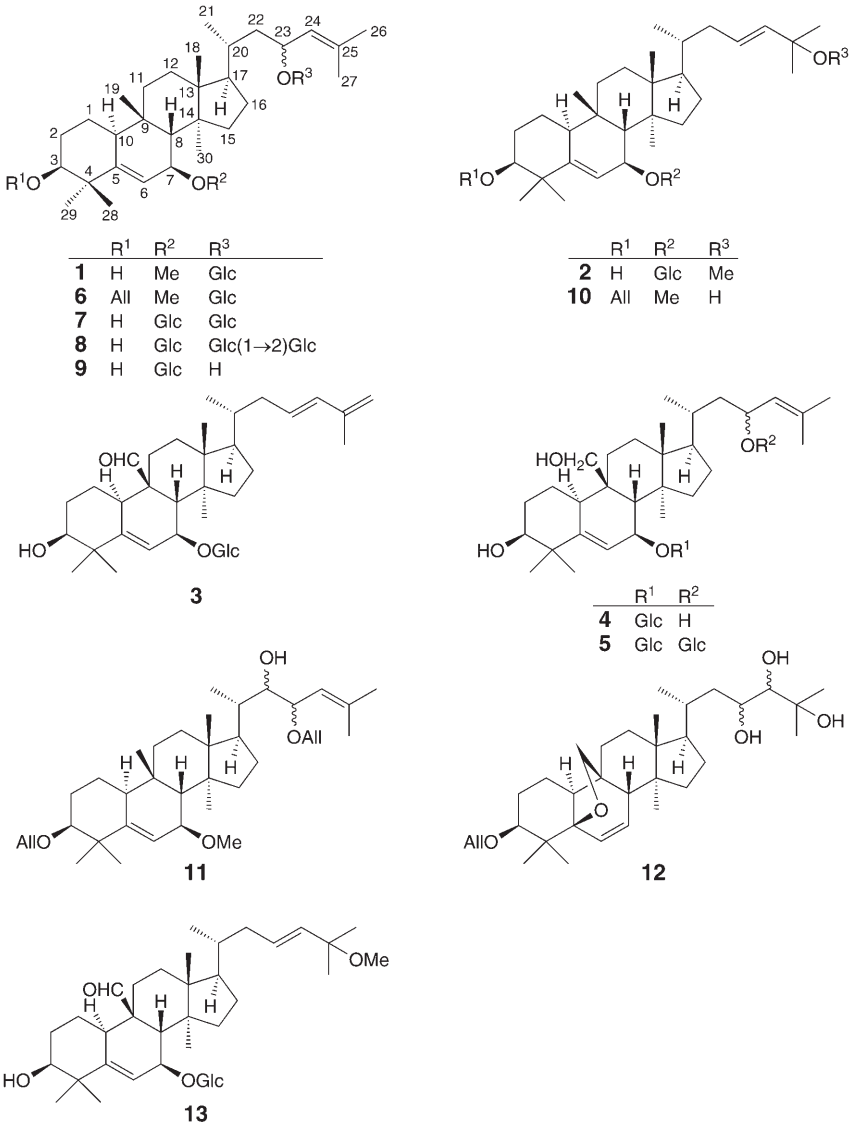
Eight new cucurbitane glycosides, kuguaglycosides A – H (**1** – **8**, resp.), together with five known analogues, 3β,23-dihydroxycucurbita-5,24-dien-7β-yl β-D-glucopyranoside (**9**), karaviloside III (**10**), karaviloside V (**11**), karaviloside XI (**12**), and momordicoside K (**13**), were isolated from the root of *Momordica charantia* L. The structures of the new compounds were determined on the basis of spectroscopic and chemical methods.

Introduction. – *Momordica charantia* L. (Cucurbitaceae) is widely distributed in Asian countries and cultivated as a vegetable crop. Its fruit, called kugua in Chinese or bitter melon in English, is a popular vegetable in China, and is becoming increasingly popular in western countries as a food supplement to lower blood glucose [1]. The root of *M. charantia* has been used in China for treating a number of diseases, such as toothache, diarrhea, furuncle, and diabetes [2]. Cucurbitane glycosides, pentacyclic triterpene glycosides, and sterol glycosides have been reported from the fruit of this species [3 – 10]. Some cucurbitacins isolated from fruit were reported to show blood hypoglycaemic effects in diabetic mice [7] or inhibitory effects in both 7,12-dimethylbenz[*a*]anthracene (DMBA)- and peroxynitrite (PN)-induced mouse skin carcinogenesis tests [8]. Momorcharaside A, a cucurbitane glycoside obtained from the seeds of this plant, was reported to exhibit certain inhibition of DNA and RNA syntheses in S180 tumor cells in preliminary pharmacological studies [11].

Though many phytochemical studies [3 – 18] on the fruit, leaf, vine, and seed of this plant have been carried out, there has been no report on chemical constituents of the root. In the course of our search for potential bioactive cucurbitane compounds from cucurbitaceous plants, we have examined the root of *M. charantia* and found eight new cucurbitane glycosides, named kuguaglycosides A – H (**1** – **8**), along with five known ones, 3β,23-dihydroxycucurbita-5,24-dien-7β-yl β-D-glucopyranoside (**9**) [15], karaviloside III (**10**) [6], karaviloside V (**11**) [6], karaviloside XI (**12**) [9], and momordicoside K (**13**) [4] (*Fig. 1*). This paper deals with the isolation and structural elucidation of the new cucurbitane glycosides based on spectroscopic analysis and chemical methods.

Results and Discussion. – Kuguaglycoside A (**1**) was obtained as a white powder with an optical rotation $[\alpha]_D^{24} = +12.7$ ($c = 0.6$, MeOH). The molecular formula was



	R^1	R^2	R^3
1	H	Me	Glc
6	All	Me	Glc
7	H	Glc	Glc
8	H	Glc	Glc(1→2)Glc
9	H	Glc	H

	R^1	R^2	R^3
2	H	Glc	Me
10	All	Me	H

3

	R^1	R^2
4	Glc	H
5	Glc	Glc

11 **12** **13**

Fig. 1. *Structures of compounds* **1**–**13**

determined as $C_{37}H_{62}O_8$ by HR-FAB-MS, ^{13}C-NMR, and DEPT experiments. The IR spectrum of **1** showed strong absorption bands at 3328 and 1079 cm^{-1}, which suggested the presence of OH groups. After acid hydrolysis of **1** with 5% dry HCl/MeOH, glucose was detected by TLC. In the ^{1}H-NMR spectrum (*Table 1*), the aglycone showed resonances for seven Me *singlets* at δ(H) 0.76, 0.87, 1.14, 1.18, 1.45, 1.68, and 1.77 (3 H each, *s*), one Me *doublet* at δ(H) 1.20 ($J = 6.3$ Hz), three oxygenated CH at δ(H) 3.45 (br. *d*, $J = 5.1$ Hz), 3.59 (br. *s*), and 4.95–4.97 (*m*), and a MeO group at δ(H) 3.35 (*s*).

The anomeric H-atom signal at δ(H) 4.97 (d, $J = 7.8$ Hz) suggested the presence of a β-glucopyranosyl moiety. Comparison of the ^{13}C-NMR data of **1** with those of **9** [15] showed similar signals except for those around both the C(7) and C(23) positions. The ^{13}C-NMR data for both C(7) and C(23) (*Table 2*) were shifted downfield from δ(C) 72.6 and 65.3 in **9** to δ(C) 77.7 and 75.7 in **1**, respectively, which suggested that the sugar unit is located at C(23), and the MeO group is positioned at C(7). In the HMBC spectrum of **1**, long-range correlations were observed from the anomeric H-atom at δ(H) 4.97 to C(23), as well as from the MeO *singlet* at δ(H) 3.35 to C(7). Consequently, the structure of kuguaglycoside A was determined to be 3β-hydroxy-7β-methoxycucurbita-5,24-dien-23-yl β-glucopyranoside.

Kuguaglycoside B (**2**) was isolated as a white powder. The molecular formula $C_{37}H_{62}O_8$ was deduced from the quasimolecular ion peak at m/z 633.4373 $[M-H]^-$ in the negative HR-FAB-MS as well as the analysis of NMR data. Careful comparison of the ^{1}H- and ^{13}C-NMR data of **2** (*Tables 1–3*) with those of karaviloside III (**10**) [6], revealed that the two compounds showed similarities except for a β-glucopyranosyl moiety in **2** instead of a β-allopyranosyl moiety in **10**, as well as those around the positions of C(3), C(7), and C(25). In the HMBC spectrum of **2**, long-range correlations observed from the anomeric H-atom signal at δ(H) 5.11 (d, $J = 7.7$ Hz) to C(7) (δ(C) 72.4), and from the MeO *singlet* at δ(H) 3.20 to C(25) (δ(C) 74.9), manifested that the MeO group was located at C(25), and the sugar unit was attached at C(7). Thus, the structure of **2** was elucidated as 3β-hydroxy-25-methoxycucurbita-5,23-dien-7β-yl β-glucopyranoside.

Kuguaglycoside C (**3**) was obtained as colorless needles. The molecular formula was determined as $C_{36}H_{56}O_8$ from the negative HR-FAB-MS and NMR data. The ^{1}H-NMR spectrum of **3** (*Table 1*) showed the presence of four Me *singlets* at δ(H) 0.75, 0.85, 1.13, 1.43 (3 H each, s), a Me *doublet* at δ(H) 0.92 ($J = 5.6$ Hz), a Me group attached to an olefinic C-atom at δ(H) 1.89 (s), a terminal CH_2 group at δ(H) 4.97 (1 H, s) and 5.03 (1 H, s), a CHO group at δ(H) 10.49 (1 H, s), and the anomeric H-atom of a sugar unit at δ(H) 4.97 (d, $J = 7.7$ Hz). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with the literature data of (23*E*)-3β-hydroxy-7β-methoxycucurbita-5,23,25-trien-19-al [17] showed similarities except that a MeO group at C(7) in the known compound was replaced by a β-(glucopyranosyloxy) function in **3**. The upfield shift of the C-atom signal for C(7) (– 3.7) in **3** indicated that the sugar unit was attached at C(7). The HMBC correlation observed from the anomeric H-atom H–C(1′) to C(7) also supported the above suggestion. Consequently, the structure of **3** was elucidated as 7β-(β-glucopyranosyloxy)-3β-hydroxycucurbita-5,23,25-trien-19-al.

Kuguaglycoside D (**4**), obtained as a white amorphous powder, liberated glucose by acid hydrolysis. Its molecular formula $C_{36}H_{60}O_9$, determined from the negative HR-FAB-MS, had one more O-atom than that of **9** [15]. Comparison of the 1D-NMR data of **4** with those of **9** revealed that they were quite similar except for a CH_2OH signal at δ(C) 65.3 in **4** instead of a Me group at δ(C) 29.3 for C(19) in **9**. Moreover, the HMBC correlations of **4** were observed from H–C(19) (δ(H) 3.53–3.55 (m) and 4.30–4.31 (m)) to C(8) (δ(C) 39.1), C(9) (δ(C) 39.1), and C(10) (δ(C) 39.0). Presence of an OH group at C(19) was also confirmed by the correlation of H–C(19) with H–C(8) (δ(H) 3.00, br. s) in the NOESY spectrum. Therefore, the structure of **4** was determined to be 3β,19,23-trihydroxycucurbita-5,24-dien-7β-yl β-glucopyranoside.

Table 1. *^{1}H-NMR Data for Compounds* **1**–**4** *in C_5D_5N*[a]). δ in ppm, J in Hz.

	1[b])	**2**[c])	**3**[b])	**4**[b])
CH_2(1)	1.68–1.71 (*m*), 1.59–1.61 (*m*)	1.66–1.69 (*m*), 1.59–1.60 (*m*)	1.93–1.95 (*m*), 1.68–1.70 (*m*)	1.89–1.91 (*m*), 1.65–1.68 (*m*)
CH_2(2)	2.00–2.03 (*m*), 1.96–1.99 (*m*)	2.01–2.03 (*m*), 1.95–1.97 (*m*)	2.01–2.03 (*m*), 1.90–1.95 (*m*)	1.95–1.98 (*m*), 1.88–1.91 (*m*)
H–C(3)	3.59 (br. *s*)	3.79 (br. *s*)	3.79 (br. *s*)	3.74 (br. *s*)
H–C(6)	5.99 (*d*, J = 4.8)	6.03 (*d*, J = 4.6)	6.17 (*d*, J = 5.0)	6.06 (*d*, J = 4.4)
H–C(7)	3.45 (br. *d*, J = 5.1)	4.58 (br. *d*, J = 4.6)	4.59 (br. *d*, J = 5.0)	4.51 (br. *d*, J = 5.2)
H–C(8)	2.14 (br. *s*)	2.50 (br. *s*)	2.53 (br. *s*)	3.00 (br. *s*)
H–C(10)	2.38–2.40 (*m*)	2.41–2.43 (*m*)	2.62–2.64 (*m*)	2.55–2.57 (*m*)
CH_2(11)	1.74–1.77 (*m*), 1.50–1.53 (*m*)	1.76–1.79 (*m*), 1.52–1.54 (*m*)	2.59–2.63 (*m*), 1.54–1.56 (*m*)	2.29–2.33 (*m*), 1.59–1.62 (*m*)
CH_2(12)	1.62–1.64 (*m*), 1.46–1.48 (*m*)	1.65–1.68 (*m*), 1.49–1.52 (*m*)	1.66–1.70 (*m*)	1.72–1.75 (*m*), 1.53–1.55 (*m*)
CH_2(15)	1.30–1.34 (*m*)	1.32–1.34 (*m*)	1.35–1.37 (*m*)	1.34–1.37 (*m*)
CH_2(16)	1.84–1.87 (*m*), 1.47–1.49 (*m*)	1.88–1.90 (*m*), 1.46–1.48 (*m*)	1.91–1.94 (*m*), 1.42–1.44 (*m*)	1.92–1.94 (*m*), 1.40–1.42 (*m*)
H–C(17)	1.51 (overlapped)	1.51 (overlapped)	1.52 (overlapped)	1.60 (overlapped)
Me(18)	0.87 (*s*)	0.87 (*s*)	0.85 (*s*)	1.22 (*s*)
Me(19), H–C(19)O, or CH_2(19)	1.18 (*s*)	1.41 (*s*)	10.49 (*s*)	4.30–4.31 (*m*), 3.53–3.55 (*m*)
H–C(20)	2.11–2.13 (*m*)	1.48 (overlapped)	1.50 (overlapped)	2.14–2.16 (*m*)
Me(21)	1.20 (*d*, J = 6.3)	0.96 (*d*, J = 5.2)	0.92 (*d*, J = 5.6)	1.18 (*d*, J = 6.2)
CH_2(22)	2.03–2.05 (*m*), 1.23–1.25 (*m*)	2.41–2.43 (*m*), 1.95–1.96 (*m*)	2.26–2.28 (*m*), 1.88–1.90 (*m*)	2.03–2.05 (*m*), 1.24–1.26 (*m*)
H–C(23)	4.95–4.97 (*m*)	5.62–5.64 (*m*)	5.75 (*ddd*, J = 5.4, 8.3, 15.6)	4.82–4.84 (*m*)
H–C(24)	5.63 (*d*, J = 8.7)	5.55 (*d*, J = 15.8)	6.30 (*d*, J = 15.6)	5.63 (*d*, J = 8.2)
Me(26) or CH_2(26)	1.68 (*s*)	1.31 (*s*)	5.03 (*s*), 4.97 (*s*)	1.73 (*s*)
Me(27)	1.77 (*s*)	1.31 (*s*)	1.89 (*s*)	1.74 (*s*)
Me(28)	1.45 (*s*)	1.41 (*s*)	1.43 (*s*)	1.38 (*s*)
Me(29)	1.14 (*s*)	1.10 (*s*)	1.13 (*s*)	1.12 (*s*)
Me(30)	0.76 (*s*)	0.71 (*s*)	0.75 (*s*)	0.82 (*s*)
MeO	3.35 (*s*)	3.20 (*s*)		
Glc:				
H–C(1′)	4.97 (*d*, J = 7.8)	5.11 (*d*, J = 7.7)	4.97 (*d*, J = 7.7)	5.07 (*d*, J = 7.8)
H–C(2′)	4.02–4.04 (*m*)	4.09–4.11 (*m*)	4.01 (*t*-like, J = 8.3)	4.06 (*t*-like, J = 8.0)
H–C(3′)	4.22–4.24 (*m*)	4.34–4.36 (*m*)	4.26–4.28 (*m*)	4.31–4.33 (*m*)
H–C(4′)	4.20–4.22 (*m*)	4.29–4.31 (*m*)	4.23–4.25 (*m*)	4.28–4.30 (*m*)
H–C(5′)	3.88–3.90 (*m*)	4.04–4.06 (*m*)	4.02–4.04 (*m*)	4.00–4.02 (*m*)
CH_2(6′)	4.47 (*d*, J = 9.7), 4.33–4.35 (*m*)	4.65 (br. *d*, J = 10.6), 4.46 (*dd*, J = 5.1, 11.6);	4.63 (*dd*, J = 2.0, 11.8), 4.41 (*dd*, J = 5.6, 11.8)	4.59 (br. *d*, J = 10.2), 4.40 (*dd*, J = 5.3, 11.8)

[a]) Assignments were established with HSQC, HMBC, COSY, and ROESY spectra. [b]) Recorded at 400 MHz. [c]) Recorded at 500 MHz.

Table 2. *^{13}C-NMR Data of the Aglycone Moieties of* **1**–**8** *in C_5D_5N*[a]). δ in ppm.

Position	**1**[b])	**2**[c])	**3**[b])	**4**[b])	**5**[b])	**6**[c])	**7**[c])	**8**[c])
1	21.9 (*t*)	21.8 (*t*)	21.9 (*t*)	20.5 (*t*)	20.5 (*t*)	22.7 (*t*)	21.8 (*t*)	21.8 (*t*)
2	30.3 (*t*)	30.2 (*t*)	29.9 (*t*)	30.4 (*t*)	30.4 (*t*)	28.9 (*t*)	30.2 (*t*)	30.2 (*t*)
3	76.2 (*d*)	76.1 (*d*)	75.7 (*d*)	76.1 (*d*)	76.1 (*d*)	87.9 (*d*)	76.1 (*d*)	76.2 (*d*)
4	42.1 (*s*)	42.0 (*s*)	42.0 (*s*)	42.1 (*s*)	42.1 (*s*)	42.1 (*s*)	42.0 (*s*)	42.0 (*s*)
5	148.2 (*s*)	148.4 (*s*)	149.9 (*s*)	148.9 (*s*)	148.9 (*s*)	148.0 (*s*)	148.4 (*s*)	148.5 (*s*)
6	119.7 (*d*)	121.0 (*d*)	122.4 (*d*)	121.0 (*d*)	121.0 (*d*)	119.1 (*d*)	121.1 (*d*)	121.1 (*d*)
7	77.7 (*d*)	72.4 (*d*)	72.0 (*d*)	72.8 (*d*)	72.9 (*d*)	77.7 (*d*)	72.6 (*d*)	72.1 (*d*)
8	48.4 (*d*)	48.4 (*d*)	45.4 (*d*)	39.1 (*d*)	39.1 (*d*)	48.7 (*d*)	48.5 (*d*)	48.6 (*d*)
9	35.1 (*s*)	34.5 (*s*)	50.5 (*s*)	39.1 (*s*)	39.2 (*s*)	34.3 (*s*)	34.5 (*s*)	35.1 (*s*)
10	39.4 (*d*)	39.3 (*d*)	36.8 (*d*)	39.0 (*d*)	39.0 (*d*)	39.4 (*d*)	39.3 (*d*)	39.3 (*d*)
11	28.1 (*t*)	28.0 (*t*)	22.7 (*t*)	27.0 (*t*)	27.0 (*t*)	28.0 (*t*)	28.2 (*t*)	28.2 (*t*)
12	33.1 (*t*)	33.0 (*t*)	29.4 (*t*)	28.4 (*t*)	28.3 (*t*)	32.8 (*t*)	33.1 (*t*)	33.1 (*t*)
13	46.5 (*s*)	46.3 (*s*)	45.9 (*s*)	46.3 (*s*)	46.3 (*s*)	46.4 (*s*)	46.5 (*s*)	46.5 (*s*)
14	48.4 (*s*)	48.2 (*s*)	48.2 (*s*)	47.8 (*s*)	47.8 (*s*)	48.3 (*s*)	48.3 (*s*)	48.0 (*s*)
15	34.5 (*t*)	34.5 (*t*)	35.0 (*t*)	35.3 (*t*)	35.4 (*t*)	34.9 (*t*)	34.9 (*t*)	34.5 (*t*)
16	30.7 (*t*)	30.5 (*t*)	27.8 (*t*)	30.5 (*t*)	30.5 (*t*)	30.6 (*t*)	30.7 (*t*)	30.8 (*t*)
17	51.5 (*d*)	50.3 (*d*)	50.5 (*d*)	51.5 (*d*)	51.5 (*d*)	51.4 (*d*)	51.4 (*d*)	51.5 (*d*)
18	15.6 (*q*)	15.7 (*q*)	15.1 (*q*)	15.0 (*q*)	15.1 (*q*)	15.5 (*q*)	15.7 (*q*)	15.9 (*q*)
19	29.3 (*q*)	29.5 (*q*)	207.6 (*d*)	65.3 (*t*)	65.6 (*t*)	29.2 (*q*)	29.4 (*q*)	29.4 (*q*)
20	32.9 (*d*)	36.5 (*d*)	36.9 (*d*)	33.1 (*d*)	32.9 (*d*)	32.9 (*d*)	32.9 (*d*)	33.0 (*d*)
21	19.7 (*q*)	19.1 (*q*)	19.1 (*q*)	19.3 (*q*)	19.8 (*q*)	19.7 (*q*)	19.8 (*q*)	20.1 (*q*)
22	44.0 (*t*)	39.8 (*t*)	40.1 (*t*)	45.6 (*t*)	44.1 (*t*)	43.9 (*t*)	44.1 (*t*)	44.3 (*t*)
23	75.7 (*d*)	128.5 (*d*)	129.8 (*d*)	65.6 (*d*)	75.7 (*d*)	75.8 (*d*)	75.2 (*d*)	75.8 (*d*)
24	129.2 (*d*)	137.6 (*d*)	134.8 (*d*)	130.8 (*d*)	129.2 (*d*)	129.1 (*d*)	129.3 (*d*)	129.0 (*d*)
25	132.3 (*s*)	74.9 (*s*)	142.6 (*s*)	132.0 (*s*)	132.1 (*s*)	132.3 (*s*)	132.1 (*s*)	132.2 (*s*)
26	26.0 (*q*)	26.5 (*q*)	114.7 (*t*)	25.8 (*q*)	25.9 (*q*)	25.9 (*q*)	25.9 (*q*)	25.9 (*q*)
27	18.4 (*q*)	26.5 (*q*)	18.9 (*q*)	18.1 (*q*)	18.4 (*q*)	18.3 (*q*)	18.4 (*q*)	18.4 (*q*)
28	26.4 (*q*)	26.1 (*q*)	26.3 (*q*)	26.4 (*q*)	26.4 (*q*)	25.9 (*q*)	26.4 (*q*)	26.5 (*q*)
29	28.5 (*q*)	28.4 (*q*)	27.4 (*q*)	28.3 (*q*)	28.3 (*q*)	29.0 (*q*)	28.5 (*q*)	28.5 (*q*)
30	18.2 (*q*)	18.0 (*q*)	18.3 (*q*)	18.4 (*q*)	18.4 (*q*)	18.1 (*q*)	18.1 (*q*)	18.1 (*q*)
MeO	56.3 (*q*)	50.2 (*q*)				56.2 (*q*)		

[a]) Assignments were established with HSQC, HMBC, COSY, and ROESY spectra. [b]) Recorded at 100 MHz. [c]) Recorded at 125 MHz.

Kuguaglycoside E (**5**) exhibited a molecular formula of $C_{42}H_{70}O_{14}$ from negative HR-FAB-MS and NMR data. Its IR spectrum revealed strong absorption bands at 3385, 1072, and 1030 cm^{-1} suggesting glycoside groups. The ^{1}H-NMR spectra showed two anomeric H-atoms at δ(H) 5.08 (*d*, $J = 7.7$ Hz, H−C(1′)) and 4.97 (*d*, $J = 7.8$ Hz, H−C(1″)), as well as a cucurbitane aglycone moiety. The ^{13}C-NMR data of the aglycone in **5** (*Table 2*) were superimporsable with those of **4**, except for a downfield shift of C(23) (+10.1), indicating that the second sugar was located at C(23) in **5**. In the HMBC spectrum of **5**, long-range correlations were observed from the anomeric H-atom at δ(H) 4.97 (H−C(1″)) to C(23) (δ(C) 75.7). Therefore, the structure of **5** was elucidated as 23-(β-glucopyranosyloxy)-3β,19-dihydroxycucurbita-5,24-dien-7β-yl β-glucopyranoside.

Table 3. *^{13}C-NMR Data of the Sugar Moieties of Compounds* **1**–**8**

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	26-*O*-Glc	7-*O*-Glc	7-*O*-Glc	7-*O*-Glc	7-*O*-Glc	3-*O*-All	7-*O*-Glc	7-*O*-Glc
1′ (*d*)	104.3	101.2	102.0	101.3	101.3	105.1	101.3	100.9
2′ (*d*)	75.5	75.3	75.1	74.9	74.9	72.1	75.2	75.1
3′ (*d*)	79.0	78.9	78.8	79.3	79.1[a])	73.4	79.0[c])	78.6[d])
4′ (*d*)	72.0	72.0	71.9	71.8	71.8	69.3	72.0	72.2[f])
5′ (*d*)	78.3	78.8	78.7	79.0	78.4[a])	75.7	78.3[c])	78.2[d])
6′ (*t*)	63.0	63.0	63.1	63.0	62.8[b])	63.3	63.0	62.8[e])
					23-*O*-Glc	23-*O*-Glc	23-*O*-Glc	23-*O*-Glc (inner)
1″ (*d*)					104.5	104.3	104.5	102.6
2″ (*d*)					75.6	75.4	75.7	82.8
3″ (*d*)					79.3[a])	79.0	79.0[c])	78.7[d])
4″ (*d*)					71.8	71.8	72.0	72.1[f])
5″ (*d*)					79.0[a])	78.4	78.6[c])	78.3[d])
6″ (*t*)					63.0[b])	63.0	63.0	63.1[e])
								23-*O*-Glc (terminal)
1‴ (*d*)								105.4
2‴ (*d*)								76.5
3‴ (*d*)								78.9[d])
4‴ (*d*)								72.2[f])
5‴ (*d*)								78.3[d])
6‴ (*t*)								63.2[e])

[a])–[f]) Assignments may be interchanged in each column.

Kuguaglycoside F (**6**) was isolated as a white powder. The molecular formula $C_{43}H_{72}O_{13}$ was deduced from the HR-FAB-MS, as well as from its ^{13}C-NMR and DEPT data. Allose and glucose were detected during the acid hydrolysis of **6**. The ^{1}H- and ^{13}C-NMR spectra exhibited signals assignable to a kuguaglycoside A (**1**) moiety and an additional glycosidic residue (δ(H) 5.32, *d*, $J = 7.9$ Hz, H–C(1′)). The ^{13}C-NMR signal for C(3) was shifted downfield from δ(C) 76.2 in **1** to δ(C) 87.9 in **6**, which indicated that the β-allopyranosyl moiety was attached at C(3). The location of the β-allopyranosyl function was substantiated by long-range HMBC correlations between the anomeric H-atom signal at δ(H) 5.32 (H–C(1′)) and C(3) (δ(H) 87.9). Thus, the structure of **6** was determined to be 23-(β-glucopyranosyloxy)-7β-methoxycucurbita-5,24-dien-3β-yl β-allopyranoside.

Kuguaglycoside G (**7**) was assigned the molecular formula $C_{42}H_{70}O_{13}$ from its HR-FAB-MS and NMR data. Acid hydrolysis of **7** liberated glucose. The ^{1}H- and ^{13}C-NMR spectra showed signals ascribable to an additional glycosidic functionality (δ(H) 4.96, *d*, $J = 7.8$ Hz, H–C(1″)), together with signals analogous to the signals of compound **9**. The ^{13}C-NMR signal for C(23) was shifted downfield from δ(C) 65.3 in **9** to δ(C) 75.2 in **7**, indicating that the additional glucopyranosyl moiety was located at C(23). This was confirmed by the HMBC correlations from the anomeric H-atom signal at δ(H) 4.96 (H–C(1″)) to C(23) (δ(C) 75.2). Consequently, the structure of **7** was elucidated as 23-(β-glucopyranosyloxy)-3β-hydroxycucurbita-5,24-dien-7β-yl β-glucopyranoside.

Kuguaglycoside H (**8**) was isolated as colorless needles, and liberated glucose during acid hydrolysis. The molecular formula was determined as $C_{48}H_{80}O_{18}$ by HR-FAB-MS and ^{13}C-NMR. The ^{1}H- and ^{13}C-NMR spectra showed signals ascribable to an additional glycosidic functionality (δ(H) 5.50, *d*, $J = 7.7$ Hz, H–C(1‴)) together with analogous signals to kuguaglycoside G (**7**). Comparison of the ^{13}C-NMR spectrum of **8** with the one of **7** revealed that the signal of the anomeric C-atom linked to C(23) was shifted upfield from δ(C) 104.5 in **7** to δ(C) 102.6 in **8** (*Table 3*). It was reported [19] that β-D-glucosylation at the 2-OH group of a glucosyl moiety (β-sophoroside residue) results in an upfield shift of this anomeric C-atom signal by *ca.* 2 ppm. Based on this report, as well as the ^{13}C-NMR spectroscopic data, the three anomeric C-atoms of **8** can be reasonably assigned as follows: the signal at δ(C) 102.6 to the C-atom of 23-*O*-β-sophorosyl linkage, the signal at δ(C) 105.4 to the terminal β-glucoside of the β-sophoroside, and the signal at δ(C) 100.9 to 7-*O*-β-glucoside, respectively. Furthermore, in the HMBC experiment on **8** (*Fig. 2*), long-range correlations were observed between the following H-atoms and C-atoms: H–C(1′) (δ(H) 5.11 (*d*, $J = 7.7$ Hz)) and C(7) (δ(C) 72.1); H–C(1″) (δ(H) 4.95 (*d*, $J = 7.5$ Hz)) and C(23) (δ(C) 75.8); H–C(1‴) (δ(H) 5.50 (*d*, $J = 7.7$ Hz)) and C(2″) (δ(C) 82.8). Thus, the structure of **8** was determined as 23-(β-glucopyranosyl(1 → 2)-β-glucopyranosyl)-3β-hydroxycucurbita-5,24-dien-7β-yl β-glucopyranoside.

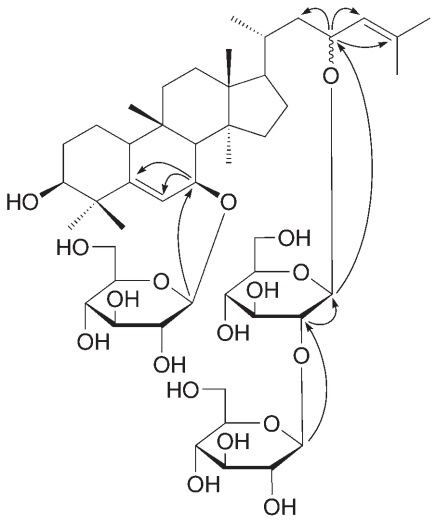


Fig. 2. *Key HMBC correlations for* **8**

Experimental Part

General. Thin-layer chromatography (TLC) was performed on plates precolated with silica gel (SiO_2) *GF*$_{254}$ (*Qingdao*); visualization under UV light and by spraying with 10% H_2SO_4 soln. followed by heating. Column chromatography (CC): SiO_2 (200–300 mesh, *Qingdao Marine Chemical*, China); *Lichroprep RP-18* (40–63 μm, *Merck*, Darmstadt, Germany); and *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*). Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Shimadzu IR-450* instrument; in cm^{-1}; KBr pellets. NMR Spectra: *Bruker AV-400* or *DRX-500* instruments; chemical

shifts (δ) in ppm; TMS as the internal standard; J in Hz. FAB-MS (negative-ion mode; glycerol matrix) and HR-FAB-MS: *VG-AUTOSPEC-3000* spectrometer; in m/z (rel. int. in % of the base peak).

Plant Material. The plants were collected at Dahanying Village, Anning County, Yunnan Province of China, in August 2005. The plant material was identified by Prof. *Shu-Kun Chen*, and a specimen (No. KIB 2005-8-25) has been deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. Air-dried root of *M. charantia* (1.9 kg) was extracted 3 times with MeOH (each 5 l) at 60° for a total of 18 h. After removal of the solvent under reduced pressure, a residue (160 g) was obtained. This residue was dissolved in H_2O (2 l), and then extracted successively with AcOEt (1 l × 3) and BuOH (1 l × 4). The BuOH extract (90 g) was subjected to CC (SiO_2) and eluted with a gradient system of $CHCl_3$/MeOH (1:0, 30:1, 20:1, 10:1, 5:1, 2:1, 0:1) to yield seven fractions. *Fr. III* (25 g) was chromatographed over SiO_2 with $CHCl_3$/MeOH (20:1, 16:1) to afford fractions *A* and *B*. Repeated chromatography over *RP-18* (MeOH/H_2O 1:1 → 6:4) and *Sephadex LH-20* (MeOH) afforded **1** (15 mg), **2** (12 mg), and **10** (20 mg) from *Fr. A*, and **3** (9 mg), **9** (31 mg), and **13** (22 mg) from *Fr. B*. Compounds **4** (99 mg) and **12** (302 mg) were isolated and purified from *Fr. IV* (10 g) by repeated CC (SiO_2) eluted with $CHCl_3$/MeOH (15:1, 12:1). *Fr. V* (20 g) was applied to CC (SiO_2) eluted with $CHCl_3$/MeOH (9:1, 6:1) and purified over *Sephadex LH-20* with MeOH as eluent to afford compounds **5** (93 mg), **6** (19 mg), **7** (124 mg), and **11** (48 mg). Compound **8** (50 mg) was obtained from *Fr. VI* (6 g) by repeated CC (SiO_2) eluted with $CHCl_3$/MeOH (3:1).

Acid Hydrolysis of **1**–**8**. To compounds **1**–**8** (each 2 mg) was added 5% dry HCl/MeOH (5 ml), and the mixture was heated at 80° for 4 h, resp. After extraction with $CHCl_3$/H_2O (1:1), the water-soluble part was neutralized with Na_2CO_3. Sugars in the aq. hydrolysate were identified with authentic samples by TLC in AcOEt/^{i}PrOH/Me_2CO/H_2O (20:10:7:6; spots were visualized by spraying with 10% H_2SO_4 soln., followed by heating). Glucose (R_f 0.51) was found in **1**, **2**, **3**, **4**, **5**, **7**, and **8**. Both glucose and allose (R_f 0.48) were detected in **6**.

Kuguaglycoside A (= *3β-Hydroxy-7β-methoxycucurbita-5,24-dien-23-yl β-Glucopyranoside*; **1**). White amorphous powder. $[\alpha]_D^{24} = +12.7$ ($c = 0.6$, MeOH). IR (KBr): 3328, 2955, 2875, 1658, 1454, 1381, 1079, 1031, 980, 895. ^{1}H-NMR (C_5D_5N, 400 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 100 MHz): *Tables 2* and *3*. FAB-MS (neg.): 633 ($[M-H]^-$). HR-FAB-MS: 633.4335 ($[M-H]^-$, $C_{37}H_{61}O_8^-$; calc. 633.4366).

Kuguaglycoside B (= *3β-Hydroxy-25-methoxycucurbita-5,23-dien-7β-yl β-Glucopyranoside*; **2**). White powder. $[\alpha]_D^{24} = +5.6$ ($c = 0.3$, MeOH). IR (KBr): 3407, 2937, 2866, 1467, 1451, 1085, 1031, 979, 941. ^{1}H-NMR (C_5D_5N, 500 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 125 MHz): *Tables 2* and *3*. FAB-MS (neg.): 633 ($[M-H]^-$). HR-FAB-MS: 633.4373 ($[M-H]^-$, $C_{37}H_{61}O_8^-$; calc. 633.4366).

Kuguaglycoside C (= *7β-(β-Glucopyranosyloxy)-3β-hydroxycucurbita-5,23,25-trien-19-al*; **3**). Colorless needles (MeOH). $[\alpha]_D^{24} = -27.9$ ($c = 0.2$, MeOH). UV (MeOH): 223 (4.82), 217 (4.83), 211 (5.19), 206 (5.19). IR (KBr): 3380, 2950, 2874, 1383, 1076, 1034, 1705, 965, 941. ^{1}H-NMR (C_5D_5N, 400 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 100 MHz): *Tables 2* and *3*. FAB-MS (neg.): 615 $[M-H]^-$. HR-FAB-MS: 615.3900 ($[M-H]^-$, $C_{36}H_{55}O_8^-$; calc. 615.3896).

Kuguaglycoside D (= *3β,19,23-Trihydroxycucurbita-5,24-dien-7β-yl β-Glucopyranoside*; **4**). White amorphous powder. $[\alpha]_D^{24} = +6.5$ ($c = 0.7$, MeOH). IR (KBr): 3401, 2943, 2917, 2875, 1460, 1381, 1103, 1071, 1029, 948. ^{1}H-NMR (C_5D_5N, 400 MHz): *Table 1*. ^{13}C-NMR (C_5D_5N, 100 MHz): *Tables 2* and *3*. FAB-MS (neg.): 635 ($[M-H]^-$). HR-FAB-MS: 635.4134 ($[M-H]^-$, $C_{36}H_{59}O_9^-$; calc. 635.4159).

Kuguaglycoside E (= *23-(β-Glucopyranosyloxy)-3β,19-dihydroxycucurbita-5,24-dien-7β-yl β-Glucopyranoside*; **5**). White amorphous powder. $[\alpha]_D^{24} = +3.9$ ($c = 0.5$, MeOH). IR (KBr): 3385, 2918, 2873, 1652, 1456, 1381, 1072, 1030, 840. ^{1}H-NMR (C_5D_5N, 400 MHz): 0.77, 1.11, 1.18, 1.36, 1.67, 1.74 (6*s*, Me(30), Me(18), Me(29), Me(28), Me(26), Me(27)); 1.18 (overlapped, Me(21)); 1.58 (overlapped, H–C(17)); 2.19 (overlapped, H–C(20)); 2.56–2.57 (*m*, H–C(10)); 3.07 (br. *s*, H–C(8)); 3.76 (br. *s*, H–C(3)); 3.53–3.55 (*m*, H_β–C(19); 4.31–4.33 (*m*, H_α–C(19)); 4.49 (*d*, $J = 5.3$, H–C(7)); 4.91–4.93 (*m*, H–C(23)); 4.97 (*d*, $J = 7.8$, H–C(1″)); 5.08 (*d*, $J = 7.7$, H–C(1′)); 5.62 (*d*, $J = 8.6$, H–C(24)); 6.01 (*d*, $J = 5.1$, H–C(6)). ^{13}C-NMR (C_5D_5N, 100 MHz): *Tables 2* and *3*. FAB-MS (neg.): 797 ($[M-H]^-$). HR-FAB-MS: 797.4697 ($[M-H]^-$, $C_{42}H_{69}O_{14}^-$; calc. 797.4687).

Kuguaglycoside F (= *23-(β-Glucopyranosyloxy)-7β-methoxycucurbita-5,24-dien-3β-yl β-Allopyranoside*; **6**). White powder. $[\alpha]_D^{24} = +4.2$ ($c = 0.5$, MeOH). IR (KBr): 3324, 2940, 2872, 1658, 1455, 1383, 1079, 1034, 979, 934. ^{1}H-NMR (C_5D_5N, 500 MHz): 0.69, 0.91, 1.07, 1.09, 1.55, 1.61, 1.76 (7*s*, Me(30),

Me(18), Me(29), Me(19), Me(28), Me(26), Me(27)); 1.19 (*d*, *J* = 6.1, Me(21)); 1.58 (overlapped, H–C(17)); 2.18 (overlapped, H–C(20)); 2.14 (overlapped, H–C(8)); 2.30–2.31 (*m*, H–C(10)); 3.27 (*s*, MeO); 3.38 (*d*, *J* = 5.0, H–C(7)); 3.64 (br. *s*, H–C(3)); 4.97–4.99 (*m*, H–C(23)); 4.99 (*d*, *J* = 7.8, H–C(1″)); 5.32 (*d*, *J* = 7.9, H–C(1′)); 5.63 (*d*, *J* = 8.7, H–C(24)); 5.91 (*d*, *J* = 4.9, H–C(6)). ^{13}C-NMR (C_5D_5N, 125 MHz): *Tables 2* and *3*. FAB-MS (neg.): 795 ($[M-H]^-$). HR-FAB-MS: 795.4893 ($[M-H]^-$, $C_{43}H_{71}O_{13}^-$; calc. 795.4894).

Kuguaglycoside G (=*23-(β-Glucopyranosyloxy)-3β-hydroxycucurbita-5,24-dien-7β-yl β-Glucopyranoside*; **7**). White powder. $[\alpha]_D^{24} = +4.4$ (*c* = 0.7, MeOH). IR (KBr): 3431, 2923, 2873, 1645, 1460, 1382, 1079, 1020, 938. ^{1}H-NMR (C_5D_5N, 500 MHz): 0.70, 0.94, 1.10, 1.36, 1.41, 1.67, 1.76 (7*s*, Me(30), Me(18), Me(29), Me(19), Me(28), Me(26), Me(27)); 1.17 (*d*, *J* = 6.1, H–C(21)); 1.54 (overlapped, H–C(17)); 2.17 (overlapped, H–C(20)); 2.41 (overlapped, H–C(10)); 2.46 (br. *s*, H–C(8)); 3.78 (br. *s*, H–C(3)); 4.52 (*d*, *J* = 5.2, H–C(7)); 4.92–4.94 (*m*, H–C(23)); 4.96 (*d*, *J* = 7.8, H–C(1″)); 5.02 (*d*, *J* = 7.7, H–C(1′)); 5.62 (*d*, *J* = 8.4, H–C(24)); 6.01 (*d*, *J* = 5.0, H–C(6)). ^{13}C-NMR (C_5D_5N, 125 MHz): *Tables 2* and *3*. FAB-MS (neg): 781 ($[M-H]^-$). HR-FAB-MS: 781.4739 ($[M-H]^-$, $C_{42}H_{69}O_{13}^-$; calc. 781.4739).

Kuguaglycoside H (=*23-(β-Glucopyranosyl(1→2)-β-glucopyranosyl)-3β-hydroxycucurbita-5,24-dien-7β-yl β-Glucopyranoside*; **8**). Colorless needles (MeOH). $[\alpha]_D^{24} = +3.2$ (*c* = 0.3, MeOH). IR (KBr): 3489, 2924, 2878, 1647, 1455, 1384, 1077, 1036, 938. ^{1}H-NMR (C_5D_5N, 500 MHz): 0.68, 0.99, 1.10, 1.34, 1.43, 1.64, 1.71 (7*s*, Me(30), Me(18), Me(29), Me(19), Me(28), Me(26), Me(27)); 1.16 (*d*, *J* = 6.4, Me(21)); 1.53 (overlapped, H–C(17)); 2.16 (overlapped, H–C(20)); 2.41–2.42 (*m*, H–C(10)); 2.47 (br. *s*, H–C(8)); 3.79 (br. *s*, H–C(3)); 4.58 (*d*, *J* = 5.1, H–C(7)); 4.85–4.87 (*m*, H–C(23)), 4.95 (*d*, *J* = 7.5, H–C(1″)); 5.11 (*d*, *J* = 7.7, H–C(1′)); 5.50 (*d*, *J* = 7.7, H–C(1‴)); 5.63 (*d*, *J* = 8.1, H–C(24)); 6.01 (*d*, *J* = 5.0, H–C(6)). ^{13}C-NMR (C_5D_5N, 125 MHz): *Tables 2* and *3*. FAB-MS (neg.): 943 ($[M-H]^-$). HR-FAB-MS: 943.5279 ($[M-H]^-$, $C_{48}H_{79}O_{18}^-$; calc. 943.5266).

The authors are grateful to the *Chinese Academy of Sciences* (XiBu ZhiGuang Project), Special Project of High-Tech of Yunnan Development and Reform Commission, and the *State Key Laboratory Foundation* for financial support.

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Received October 15, 2007