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STRUCTURES OF NEW CUCURBITANE-TYPE TRITERPENES AND GLYCOSIDES, KARAVILAGENINS D AND E, AND KARAVILOSIDES VI, VII, VIII, IX, X, AND XI, FROM THE FRUIT OF *MOMORDICA CHARANTIA*

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Abstract — Two new cucurbitane-type triterpenes, karavilagenins D and E, and six new cucurbitane-type triterpene glycosides, karavilosides VI, VII, VIII, IX, X, and XI, were isolated from the fruit of *Momordica charantia* L. (Cucurbitaceae) cultivated in Sri Lanka. Their structures were elucidated on the basis of chemical and physicochemical evidence.

The Cucurbitaceae plant *Momordica* (*M.*) *charantia* L. has been widely cultivated in India and tropical Asian countries. The fruit of this plant has been used not only as a vegetable but also as a traditional medicine for rheumatism, gout, diabetes, and diseases of liver in Sri Lanka, India, China, and Southeast Asian countries. The alcoholic extract from the fruit of *M. charantia* originated in Sri Lanka was reported to inhibit the increase of serum glucose levels in glucose-loaded rats.[1] In addition, many cucurbitane-type triterpene glycosides were isolated from the fruit of this plant originated in Japan and Taiwan.[2–9] In previous studies, we have reported the isolation and structure elucidation of eight cucurbitane-type triterpene glycosides, goyaglycosides a, b, c, d, e, f, g, and h, and three oleanane-type triterpene glycosides, goyasaponins I, II, and III, from the fresh fruit of Japanese *M. charantia*.[10] Furthermore, three new cucurbitane-type triterpenes, karavilagenins A, B, and C, and five new cucurbitane-type triterpene glycosides, karavilosides I, II, III, IV, and V, were isolated from the dried fruit of *M. charantia* cultivated in Sri Lanka.[11] As a continuing study on Sri Lanka *M. charantia*, we have isolated two new cucurbitane-type triterpenes, karavilagenins D (**1**) and F (**2**), and five new cucurbitane-type triterpene glycosides, karavilosides VI (**3**), VII (**4**), VIII (**5**), IX (**6**), X (**7**), and XI (**8**). This paper deals with the isolation and structure elucidation of new cucurbitane-type triterpenes and glycosides from the fruit of *M. charantia*. The saponin fraction was obtained from the methanolic extract from the fruit of *M. charantia* cultivated in Nuwara Eliya, Sri Lanka, which was described previously.[11] The saponin fraction was further separated by HPLC to give **1** (0.0010%), **2** (0.0006%), **3** (0.0017%), **4** (0.0003%), **5** (0.0020%), **6** (0.0042%), **7** (0.0006%), and **8** (0.0006%).

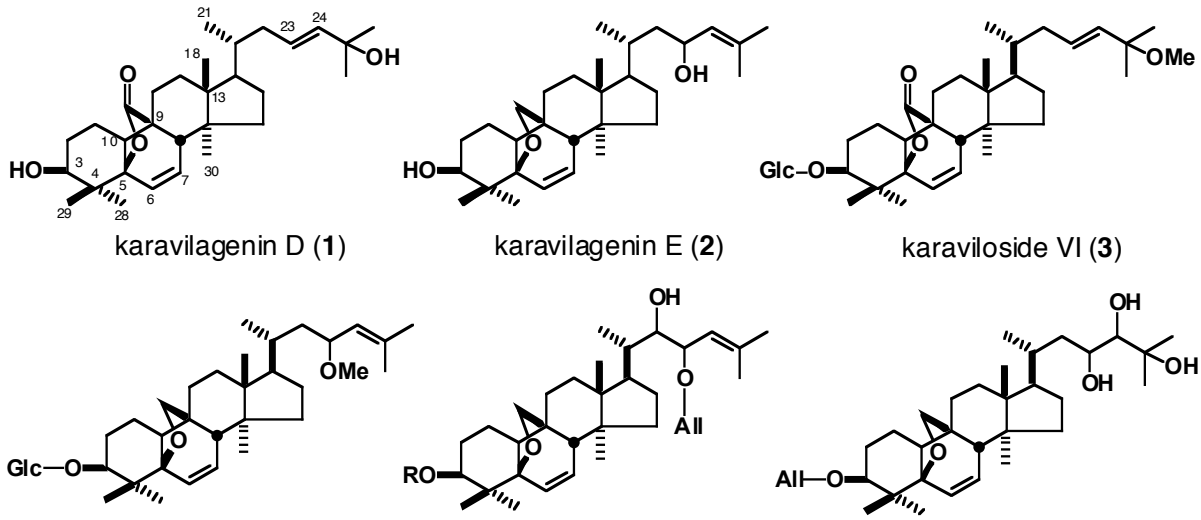


Chart 1

Structures of Karavilagenins D (1) and E (2) and Karavilosides VI–XI (3–8)

Karavilagenin D (**1**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{26}$ –94.4°). The IR spectrum of **1** showed absorption bands at 3475 and 1765 cm^{-1} suggestive of hydroxy and carbonyl functions. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 493 $(M+Na)^+$ and the molecular formula $C_{30}H_{46}O_4$ was determined by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[12] showed signals due to seven methyls [δ 0.86, 0.93, 0.95, 1.27 (3H each, all s, 29, 18, 28, 30-H_3), 0.89 (3H, d-like, 21-H_3), 1.31 (6H, s, 26, 27-H_3)], a methine bearing an oxygen function [3.42 (1H, br s, 3-H)], and four olefinic protons [5.60 (2H, m, 23, 24-H), 5.73 (1H, dd, *J*=3.3, 9.6 Hz, 7-H), 6.21 (1H, d, *J*=9.6 Hz, 6-H)]. The plane structure of **1** was determined by a detailed heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 11-H_2 and 19-C; 18-H_3 and 12, 13, 14, 17-C; 21-H_3 and 17, 20, 22-C; 23-H and 22, 24, 25-C; 24-H and 22, 23, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C (Figure 1). The stereostructure of **1** was characterized by a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: 8-H and 18-H_3; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). These findings and comparisons of ^{1}H-NMR and ^{13}C-NMR spectra of **1** with those of known cucurbitane-type triterpenes[10,11] led us to formulate the structure of karavilagenin D as 5β,19-epoxycucurbita-6,23-dien-19-on-3β,25-diol (**1**).

Karavilagenin E (**2**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{26}$ –41.0°). The IR spectrum of **2** showed absorption bands at 3475, 1055 cm^{-1} suggestive of hydroxy and ether functions. In

Table 1. ^{13}C-NMR Data (125 MHz, pyridine-d_5) for Karavilagenins D (**1**) and E (**2**) and Karavilosides VI (**3**), VII (**4**), VIII (**5**), IX (**6**), X (**7**), and XI (**8**).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C-1	18.4	17.6	19.8	18.9	17.8	18.7	18.9	19.0
C-2	26.5	27.3	26.5	27.9	27.8	27.4	27.5	27.6
C-3	75.2	76.2	84.3	85.3	76.1	85.2	85.1	85.1
C-4	37.0	37.2	38.5	39.1	37.4	38.9	39.0	39.0
C-5	85.3	85.5	85.3	85.9	87.5	85.9	86.1	86.0
C-6	131.1	131.8	132.5	134.2	132.4	133.9	134.1	134.1
C-7	133.3	131.5	133.0	130.0	131.2	129.9	130.1	130.0
C-8	44.4	52.0	45.0	52.3	52.1	52.2	52.3	52.4
C-9	51.0	45.5	50.8	45.4	45.6	45.3	45.3	45.4
C-10	39.9	38.8	40.8	40.2	39.2	40.0	40.2	40.2
C-11	21.6	23.6	21.9	23.9	23.7	23.7	23.8	23.9
C-12	29.7	30.9	30.1	31.3	31.1	31.1	31.2	31.3
C-13	45.0	45.4	45.4	45.7	45.6	45.6	45.7	45.7
C-14	47.7	48.6	48.0	49.0	48.4	48.4	48.6	49.0
C-15	33.2	33.1	33.5	33.4	33.5	33.5	33.7	33.4
C-16	27.4	28.2	27.7	28.5	27.9	27.9	28.1	28.5
C-17	50.1	50.9	50.5	51.4	46.5	46.5	46.7	51.7
C-18	14.5	14.9	14.7	15.0	14.4	14.4	14.5	15.0
C-19	181.4	79.9	182.1	80.2	79.8	79.9	80.2	80.2
C-20	36.1	32.6	36.4	32.8	40.6	40.6	40.8	32.7
C-21	18.5	18.6	18.8	19.1	14.6	14.6	14.8	19.0
C-22	39.0	44.4	39.7	43.4	76.5	76.5	76.7	43.3
C-23	125.0	65.9	128.3	75.9	80.9	80.9	81.1	67.8
C-24	139.6	129.0	137.8	128.0	124.6	124.6	124.1	79.9
C-25	70.7	133.9	74.9	134.5	135.3	135.2	135.4	73.6
C-26	29.9	18.1	26.1	25.8	26.0	26.0	26.2	27.9
C-27	29.8	25.7	26.5	18.2	18.5	18.5	18.6	27.0
C-28	19.2	20.5	20.9	21.1	20.8	20.8	21.0	21.1
C-29	23.5	24.5	24.0	25.7	24.5	25.5	25.6	25.6
C-30	20.3	20.0	19.4	20.3	20.1	20.2	20.2	20.2
23-OMe				55.6				
25-OMe			50.2					
C-1'			107.6	106.6	103.4	106.6	103.9	103.7
C-2'			74.5	75.0	72.9	75.5	73.0	73.1
C-3'			78.7	78.4	72.8	78.1	72.4	72.4
C-4'			71.8	72.0	68.9	71.8	69.3	69.4
C-5'			78.4	78.4	75.7	78.0	76.1	76.1
C-6'			63.2	63.2	62.9	62.9	63.4	63.4
C-1''						103.3	103.6	
C-2''						72.9	73.1	
C-3''						72.8	73.0	
C-4''						68.9	69.1	
C-5''						75.7	75.9	
C-6''						62.9	63.1	

the positive-ion FAB-MS of **2**, a quasimolecular ion peak was observed at *m*/*z* 457 $(M+H)^+$ and the molecular formula $C_{30}H_{49}O_3$ was determined by high-resolution MS measurement. The ^{1}H-NMR and ^{13}C-NMR spectra of **2**,[12] showed signals due to seven methyls [δ 0.86, 1.20, 1.69, 1.71 (3H each, all s, 30, 28, 27, 26-H_3), 0.90 (6H, s, 18, 29-H_3), 0.98 (3H, d, *J*=6.3 Hz, 21-H_3)], two methines bearing an oxygen function [3.41 (1H, dd-like, 3-H), 4.46 (1H, ddd, *J*=3.0, 8.3, 11.8 Hz, 23-H)], a methylene bearing an oxygen function [3.51, 3.67 (1H each, both d, *J*=8.6 Hz, 19-H_2)], and three olefinic protons [5.20 (1H, d,

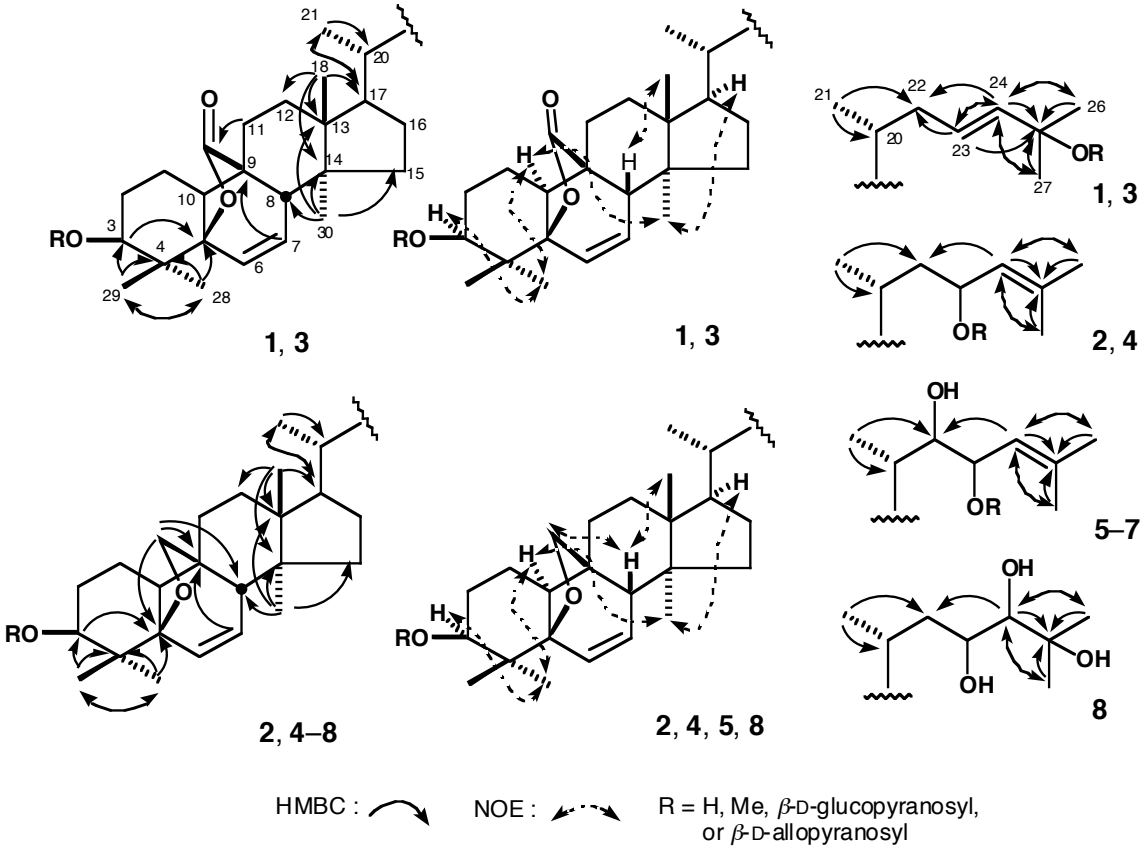


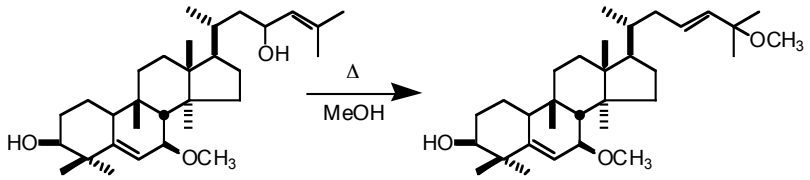
Figure 1 Selected HMBC and NOE Correlations

J=8.3 Hz, 24-H), 5.64 (1H, dd, *J*=3.6, 9.9 Hz, 7-H), 6.04 (1H, d, *J*=9.9 Hz, 6-H)]. The proton and carbon signals due to the side chain part (20-C ~ 27-C) in the ^{1}H- and ^{13}C-NMR spectra of **2** were superimposable on those of karavilagenin C (**9**),[11] while the signals due to the tetracyclic part were similar to those of 5β,19-epoxycucurbita-6,23-diene-3β,25-diol.[10] The plane structure of **2** was determined by a detailed HMBC experiment. Namely, long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_2 and 5, 8, 9-C; 21-H_3 and 17, 20, 22-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C (Figure 1). The stereostructure of **2**, except for the 23-position, was characterized by a NOESY experiment, which showed NOE correlations between the following proton pairs: 8-H and 18-H_3, 19-H_2; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). Consequently, the structure of karavilagenin E was determined to be 5β,19-epoxycucurbita-6,24-dien-3β,23ξ-diol (**2**).

Karaviloside VI (**3**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{28}$ –80.0°). The IR spectrum of **3** showed absorption bands at 3432, 1750, 1078, and 1040 cm^{-1} suggestive of carbonyl and glycosidic functions. In the negative- and positive-ion FAB-MS of **3**, quasimolecular ion peaks were observed at *m/z* 645 $(M–H)^-$, *m/z* 669 $(M+Na)^+$, and *m/z* 691 (M+2Na–H)+ and the molecular formula $C_{37}H_{58}O_9$ was determined by high-resolution MS measurement. Acid hydrolysis of **3** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.[13] The ^{1}H-NMR and ^{13}C-NMR spectra of **3**,[12] showed signals due to a β-D-glucopyranosyl moiety [δ 4.83 (d, *J*=7.9 Hz, 1'-H)] and an aglycone moiety [δ 0.83, 0.89, 0.94, 1.58 (all s,

30, 18, 29, 28-H_3), 0.95 (d-like, 21-H_3), 1.33 (s, 26, 27-H3), 3.22 (s, 25-OCH_3), 3.67 (br s, 3-H), 5.57 (d, *J*=15.9 Hz, 24-H), 5.65 (m, 6, 23-H), 6.33 (dd, *J*=2.1, 9.5 Hz, 7-H)]. The plane structure of the aglycone moiety in **3** was determined by a detailed HMBC experiment, in which long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 11-H_2 and 19-C; 18-H_3 and 12, 13, 14, 17-C; 21-H_3 and 17, 20, 22-C; 23-H and 22, 25-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C; 25-OCH_3 and 25-C; 1'-H and 3-C (Figure 1). The stereostructure of the aglycone moiety was characterized by a NOESY experiment, which showed NOE correlations between the following proton pairs: 8-H and 18-H_3; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). These findings and comparisons of ^{1}H-NMR and ^{13}C-NMR spectra of **3** with those of known cucurbitane-type triterpene glycosides (goyaglycosides, momordicosides, karavilosides I-V)[10,11] led us to formulate the structure of kalaviloside VI as 25-methoxy-5β,19-epoxycucurbita-6,23-dien-19-on-3β-ol 3-*O*-β-D-glucopyranoside (**3**).

Treatment of karavilagenin C (**9**), which was previously isolated from the fruits of Sri Lanka *M. charantia*,[11] with MeOH yielded karavilagenin A (**10**) (Scheme 1). On the basis of this finding, karaviloside VI (**3**) having the 25-methoxy group was presumed to be secondly formed from compounds having the 23-hydroxy group during the extraction or isolation procedure.



karavilagenin C (**9**) karavilagenin A (**10**)

Scheme 1

Karaviloside VII (**4**), which was obtained as a white powder with negative optical rotation ([α]$_D^{27}$ –36.3°), showed absorption bands at 3453, 1084, and 1034 cm^{-1} suggestive of a glycosidic function in the IR spectrum. The molecular formula $C_{37}H_{60}O_8$ of **4** was determined from the positive-ion FAB-MS [*m/z* 633 (M+H)$^+$] and by high-resolution MS measurement. Acid hydrolysis of **4** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.[13] The ^{1}H-NMR and ^{13}C-NMR spectra of **4**,[12] showed signals due to a β-D-glucopyranosyl moiety [δ 4.90 (1H, d, *J*=7.6 Hz, 1'-H)] and an aglycone moiety [δ 0.90, 0.80, 0.93, 1.52, 1.71, 1.75 (3H each, all s, 30, 18, 29, 28, 26, 27-H_3), 1.06 (3H, d, *J*=6.4 Hz, 21-H_3), 3.30 (3H, s, 23-OMe), 3.59, 3.73 (1H each, both d, *J*=8.0 Hz, 19-H_2), 3.69 (1H, br s, 3-H), 3.96 (1H, m, 23-H), 5.23 (1H, d-like, 24-H), 5.57 (1H, dd, *J*=4.0, 9.8 Hz, 7-H), 6.21 (1H, d-like, 6-H)]**.** The plane structure of the aglycone moiety in **4** was determined by a detailed HMBC experiment, in which long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_2 and 5, 8, 9-C; 21-H_3 and 17, 20, 22-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C; 23-OCH_3 and 23-C; 1'-H and 3-C (Figure 1). The stereostructure of the aglycone moiety, except for the 23-position, was characterized by a NOESY experiment, which showed

NOE correlations between the following proton pairs: 8-H and 18-H_3, 19-H_2; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). Consequently, the structure of karaviloside VII was determined to be 23ξ-methoxy-5β,19-epoxycucurbita-6,24-dien-3β-ol 3-*O*-β-D-glucopyranoside (**4**).

Karaviloside VIII (**5**), which was obtained as a white powder with negative optical rotation ($[\alpha]_D^{28}$ –47.4°), showed absorption bands at 3520, 1084, and 1034 cm^{-1} in the IR spectrum. The negative- and positive-ion FAB-MS of **5** showed quasimolecular ion peaks at *m/z* 633 $(M–H)^-$, *m/z* 635 $(M+H)^+$, and *m/z* 657 $(M+Na)^+$ and the molecular formula $C_{36}H_{58}O_9$ was determined by high-resolution MS measurement. Acid hydrolysis of **5** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) furnished D-allose, which was identified by GLC analysis of the thiazolidine derivative.[10,11,13] The ^{1}H-NMR and ^{13}C-NMR spectra of **5**[12] showed signals due to a β-D-allopyranosyl moiety [δ 5.53 (1H, d, *J*=7.9 Hz, 1'-H)] and an aglycone moiety [δ 0.77, 0.87, 0.89, 1.35, 1.64, 1.85 (3H each, all s, 18, 30, 29, 28, 26, 27-H_3), 1.16 (3H, d, *J*=6.9 Hz, 21-H_3), 3.51 (1H, br s, 3-H), 3.55, 3.74 (1H each, both d, *J*=8.3 Hz, 19-H_2), 4.02 (1H, dd, *J*=2.2, 7.9 Hz, 22-H), 4.64 (1H, t-like, 23-H), 5.51 (1H, m, 24-H), 5.62 (1H, dd, *J*=3.6, 9.9 Hz, 7-H), 6.14 (1H, d, *J*=9.9 Hz, 6-H)]**.** The ^{1}H- and ^{13}H-NMR signals due to the side chain part (20-C ~ 27-C) of **5** were superimposable on those of karaviloside V,[11] whereas the signals due to the tetracyclic part very resembled those of karavilagenin E (**2**). The plane structure of aglycone moiety in **5** was determined by a detailed HMBC experiment. Namely, long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_2 and 5, 8, 9-C; 21-H_3 and 17, 20, 22-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C; 1'-H and 23-C (Figure 1). The stereostructure of the aglycone moiety, except for the side chain part, was characterized by a NOESY experiment, in which NOE correlations were observed between the following proton pairs: 8-H and 18-H_3, 19-H_2; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). Consequently, the structures of karaviloside VIII was determined to be 5β,19-epoxycucurbita-6,24-dien- 3β,22ξ,23ξ-triol 23-*O*-β-D-allopyranoside (**5**).

Karavilloside IX (**6**) and X (**7**) with negative optical rotation (**6**: $[\alpha]_D^{26}$ –96.6°; **7**: $[\alpha]_D^{28}$ –80.7°) were found to have the same molecular formula, $C_{42}H_{68}O_{14}$, which was determined from the quasimolecular ion peaks in their negative-ion FAB-MS [*m/z* 795 $(M–H)^-$, *m/z* 633 $(M–C_6H_{11}O_5)^-$] and positive-ion FAB-MS [*m/z* 797 $(M+H)^+$ and *m/z* 819 $(M+Na)^+$] and by high-resolution MS measurement. The IR spectra of **6** and **7** showed absorption bands suggestive of a glycosidic function (**6**: 3453, 1082, and 1036 cm^{-1}; **7**: 3432, 1084, 1034 cm^{-1}). Acid hydrolysis of **6** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) furnished D-allose and D-glucose, which were identified by GLC analysis of their thiazolidine derivative.[10,11,13] On the other hand, **7** furnished D-allose upon acid hydrolysis. [10,11,13] The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR of **6** and **7** were superimposable on those of **5**, except for the signals due to the 3-*O*-glycoside moiety. Namely, The ^{1}H-NMR and ^{13}C-NMR spectra of **6** showed signals due to a β-D-glucopyranosyl moiety [δ 4.84 (1H, d, *J*=7.6 Hz, 1''-H)] and a β-D-allopyranosyl moiety [5.54 (d, *J*=7.9 Hz, 1'-H)] together with an aglycone moiety [δ 0.74, 0.88, 0.91, 1.48, 1.63, 1.83 (3H each, all s, 18, 30, 29, 28, 26, 27-H_3), 1.14 (3H, d, *J*=6.9 Hz, 21-H_3), 3.58, 3.73 (1H each, both d, *J*=7.9 Hz, 19-H_2), 3.65 (1H, br s, 3-H), 4.01 (1H, dd-like, 22-H), 4.68 (1H, t-like, 23-H), 5.54 (2H, m, 7, 24-H), 6.19 (1H, d, *J*=9.6 Hz, 6-H)]**.** The ^{1}H-NMR and ^{13}C-NMR spectra of **7** showed signals due to a β-D-allopyranosyl

moiety [δ 5.34 (1H, d, *J*=7.9 Hz, 1'-H) and 5.56 (d, *J*=7.9 Hz, 1''-H)] and an aglycone moiety [δ 0.75, 1.45, 1.63, 1.85 (3H each, all s, 18, 28, 26, 27-H_3), 0.89 (6H, s, 29, 30-H_3), 1.15 (3H, d, *J*=7.0 Hz, 21-H_3), 3.59, 3.76 (1H each, both d, *J*=8.3 Hz, 19-H_2), 3.62 (1H, br s, 3-H), 4.01 (1H, dd-like, 22-H), 4.63 (1H, t-like, 23-H), 5.52 (1H, d, *J*=10.2 Hz, 24-H), 5.77 (1H, dd, *J*=3.6, 9.8 Hz, 7-H), 6.18 (1H, d, *J*=9.8 Hz, 6-H)]**.** In the HMBC experiments on **6** and **7**, long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_2 and 5, 8, 9-C; 21-H_3 and 17, 20, 22-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C; 1'-H and 3-C; 1''-H and 23-C (Figure 1). Consequently, the structures of karavilosides IX and X were determined to be 23-*O*-β-D-allopyranosyl-5β,19-epoxycucurbita-6,24-dien-3β,22ξ,23ξ-triol 3-*O*-β-D-glucopyranoside (**6**) and 23-*O*-β-D-allopyranosyl-5β,19-epoxycucurbita-6,24-dien-3β,22ξ,23ξ-triol 3-*O*-β-D-allopyranoside (**7**), respectively.

Karaviloside XI (**8**, $[\alpha]_D^{27}$ –49.5°) showed absorption bands at 3432, 1084, and 1032 cm^{-1} in the IR spectrum. The molecular formula $C_{36}H_{60}O_{10}$ of **8** was determined from the negative- and positive-ion FAB-MS of **8** [*m/z* 651 $(M–H)^-$, *m/z* 653 $(M+H)^+$, and *m/z* 675 $(M+Na)^+$] and by high-resolution MS measurement. Acid hydrolysis of **8** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) furnished D-allose, which was identified by GLC analysis of the thiazolidine derivative.[10,11,13] The ^{1}H-NMR and ^{13}C-NMR spectra of **8**[12] showed signals due to a β-D-allopyranosyl moiety [δ 5.37 (1H, d, *J*=7.9 Hz, 1'-H)] and an aglycone moiety [δ 0.79, 0.88, 0.89, 1.46, 1.61, 1.63 (3H, s, 18, 30, 29, 28, 26, 27-H_3), 1.12 (3H, d, *J*=6.4 Hz, 21-H_3), 3.58, 3.76 (1H each, both d, *J*=7.9 Hz, 19-H_2), 3.52 (1H, d-like, 24-H), 3.65 (1H, br s, 3-H), 4.53 (1H, ddd-like, 23-H), 5.55 (1H, dd, *J*=3.9, 9.5 Hz, 7-H), 6.18 (1H, d, *J*=9.5 Hz, 6-H)]. The ^{1}H-NMR and ^{13}C-NMR data due to the side chain part (20-C ~ 27-C) in the aglycon of **8** were similar to those of momordicoside C[3], whereas the signals due to the tetracyclic part were superimposable on those of karaviloside X (**7**). In a HMBC experiment on **8**, long-range correlations were observed between the following protons and carbons: 3-H and 5-C; 7-H and 9-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_2 and 5, 8, 9-C; 21-H_3 and 17, 20, 22-C; 24-H and 22, 25, 26, 27-C; 26, 27-H_3 and 24, 25-C; 28, 29-H_3 and 3, 4, 5-C; 30-H_3 and 8, 13, 14, 15-C; 1'-H and 3-C (Figure 1). The NOESY experiment on **8** showed NOE correlations between the following proton pairs: 8-H and 18-H_3, 19-H_2; 28-H_3 and 3-H, 10-H; 30-H_3 and 10-H, 17-H (Figure 1). On the basis of this evidence, the structure of karaviloside XI was determined to be 5β,19-epoxycucurbita-6-en-3β,23ξ,24ξ,25-tetraol 3-*O*-β-D-allopyranoside (**8**).

EXPERIMENTAL

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (*l* = 5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica

gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Isolation of Karavilagenins D (1), and E (2), and Karavilosides VI (3), VII (4), VIII (5), IX (6), X (7), and XI (8)

Fractions 2 (37.9 g), 3 (27.6 g), and 7 (32.3 g) were obtained from the MeOH extract (4.9%) of the dried fruit of *M. charantia* L. (6.6 kg, cultivated in Nuwara Eliya, Sri Lanka) as reported previously.[11] Normal-phase silica gel column chromatography [600 g, *n*-hexane–AcOEt (1 : 1 → 1 : 5, v/v) → MeOH] of fraction 2 (33 g) gave five fractions [Fr. 2-1 (9.9 g), 2-2 (2.7 g), 2-3 (2.3 g), 2-4 (1.9 g), 2-5 (14.6 g)]. Fraction 2-2 (2.7 g) was purified by reversed-phase silica gel column chromatography [80 g, MeOH–H_2O (90 : 10, v/v) → MeOH] and HPLC [MeOH–H_2O (95 : 5, v/v)] to give karavilagenin E (**2**, 18 mg, 0.0006%). Fraction 2-4 (1.9 g) was purified by reversed-phase silica gel column chromatography [60 g, MeOH–H_2O (90 : 10, v/v) → MeOH] and HPLC [MeOH–H_2O (90 : 10, v/v)] to give karavilagenin D (**1**, 29 mg, 0.0010%). Fraction 3 (27.6 g) was separated by normal-phase silica gel column chromatography [500 g, $CHCl_3$–MeOH (50 : 1 → 50 : 3, v/v) → MeOH] to give five fractions [Fr. 3-1 (7.6 g), 3-2 (4.4 g), 3-3 (4.0 g), 3-4 (7.2 g), 3-5 (3.6 g)]. Fraction 3-3 (4.0 g) was separated by reversed-phase silica gel column chromatography [120 g, MeOH–H_2O (80 : 20 → 90 : 10, v/v) → MeOH] to give four fractions [Fr. 3-3-1 (1.5 g), 3-3-2 (569 mg), 3-3-3 (1.2 g), 3-3-4 (509 mg)]. Fraction 3-3-2 (569 mg) was purified by HPLC [1] MeOH–H_2O (90 : 10, v/v); 2] CH_3CN–H_2O (80 : 20, v/v)] to give karaviloside VI (**3**, 56 mg, 0.0017%). Fraction 3-3-3 (1.2 g) was purified by HPLC [MeOH–H2O (90 : 10, v/v)] to give karaviloside VII (**4**, 10 mg, 0.0003%). Fraction 3-4 (7.2 g) was separated by reversed-phase silica gel column chromatography [200 g, MeOH–H_2O (70 : 30 → 80 : 20 → 90 : 10 → 95 : 5, v/v) → MeOH] to give five fractions [Fr. 3-4-1 (1.8 g), 3-4-2 (403 mg), 3-4-3 (128 mg), 3-4-4 (968 mg), 3-4-5 (3.7 g)]. Fraction 3-4-2 (403 mg) was purified by HPLC [1] MeOH–H_2O (80 : 20, v/v); 2] CH_3CN-H_2O (45 : 55, v/v)] to give karaviloside XI (**8**, 19 mg, 0.0006%). Fraction 3-4-3 (128 mg) was purified by HPLC [1] MeOH–H_2O (80 : 20, v/v); 2] CH_3CN-H_2O (45 : 55, v/v)] to give karaviloside VIII (**5**, 65 mg, 0.0020%). Fraction 7 (22.3 g) was separated by reversed-phase silica gel column chromatography [600 g, MeOH–H2O (70 : 30 → 90 : 10, v/v) → MeOH] to give four fractions [Fr. 7-1 (11.3 g), 7-2 (3.7 g), 7-3 (640 mg), 7-4 (4.6 g)]. Fraction 7-2 (3.7 g) was purified by normal-phase silica gel column chromatography [100 g, $CHCl_3$-MeOH-H_2O (15 : 3 : 1, lower layer, v/v) → MeOH] to give seven fractions [Fr. 7-2-1 (320 mg), 7-2-2 (590 mg), 7-2-3 (603 mg), 7-2-4 (1.4 g), 7-2-5 (398 mg), 7-2-6 (937 mg), 7-2-7 (959 mg)]. Fraction 7-2-5 (398 mg) was purified by HPLC [MeOH-H_2O (70 : 30, v/v)] to give karaviloside X (**7**, 14 mg, 0.0006%). Fraction 7-2-6 (937 mg) was purified by HPLC [MeOH-H_2O (75 : 25, v/v)] to give karaviloside IX (**6**, 94 mg, 0.0042%).

Karavilagenin D (**1**): a white powder, $[\alpha]_D^{26}$ –94.4° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{46}O_4Na$ $(M+Na)^+$: 493.3294. Found: 493.3388. IR (KBr): 3475, 1765 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.86, 0.93, 0.95, 1.27 (3H each, all s, 29, 18, 28, 30-H_3), 0.89 (3H, d-like, 21-H_3), 1.31 (6H, s, 26, 27-H_3), 3.42 (1H, br s, 3-H), 5.60 (2H, m, 23, 24-H), 5.73 (1H, dd, *J*=3.3, 9.6 Hz, 7-H), 6.21 (1H, d, *J*=9.6 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: *m/z* 493 $(M+Na)^+$.

Karavilagenin E (**2**): a white powder, $[\alpha]_D^{26}$ –41.0° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{49}O_3$ $(M+H)^+$: 457.3682. Found: 457.3672. IR (KBr): 3475 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.86, 1.20, 1.69, 1.71 (3H each, all s, 30, 28, 26, 27-H_3), 0.90 (6H, s, 18, 29-H_3), 0.98 (3H, d, *J*=6.3 Hz, 21-H_3), 3.41 (1H, dd-like, 3-H), 3.51, 3.67 (1H each, both d, *J*=8.6 Hz, 19-H_2), 4.46 (1H, ddd, *J*=3.0, 8.3, 11.8 Hz, 23-H), 5.20 (1H, d, *J*=8.3 Hz, 24-H), 5.64 (1H, dd, *J*=3.6, 9.9 Hz, 7-H), 6.04 (1H, d, *J*=9.9 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: *m/z* 457 $(M+H)^+$.

Karaviloside VI (**3**): a white powder, $[\alpha]_D^{28}$ –80.0° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{37}H_{58}O_9Na$ $(M+Na)^+$: 669.3979. Found: 669.3992. IR (KBr): 3432, 1750, 1078, 1040 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.83, 0.89, 0.94, 1.58 (3H each, all s, 30, 18, 29, 28-H_3), 0.95 (3H, d-like, 21-H_3), 1.33 (6H, s, 26, 27-H_3), 3.22 (3H, s, 25-OMe), 3.67 (1H, br s, 3-H), 4.83 (1H, d, *J*=7.9 Hz, 1'-H), 5.57 (1H, d, *J*=15.9 Hz, 24-H), 5.65 (2H, m, 6, 23-H), 6.33 (1H, dd, *J*=2.1, 9.5 Hz, 7-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 645 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 669 $(M+Na)^+$, 691 $(M+2Na-H)^+$.

Karaviloside VII (**4**): a white powder, $[\alpha]_D^{27}$ –36.3° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{37}H_{61}O_8$ $(M+H)^+$: 633.4366. Found: 633.4361. IR (KBr): 3453, 1084, 1034 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90, 0.80, 0.93, 1.52, 1.71, 1.75 (3H each, all s, 30, 18, 29, 28, 26, 27-H_3), 1.06 (3H, d, *J*=6.4 Hz, 21-H_3), 3.30 (3H, s, 23-OMe), 3.59, 3.73 (1H each, both d, *J*=8.0 Hz, 19-H_2), 3.69 (1H, br s, 3-H), 3.96 (1H, m, 23-H), 4.90 (1H, d, *J*=7.6 Hz, 1'-H), 5.23 (1H, d-like, 24-H), 5.57 (1H, dd, *J*=4.0, 9.8 Hz, 7-H), 6.21 (1H, d-like, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 631 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 633 $(M+H)^+$.

Karaviloside VIII (**5**): a white powder, $[\alpha]_D^{28}$ –47.4° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{59}O_9$ $(M+H)^+$: 635.4159. Found: 635.4155. IR (KBr): 3520, 1084, 1034 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.77, 0.87, 0.89, 1.35, 1.64, 1.85 (3H each, all s, 18, 30, 29, 28, 26, 27-H_3), 1.16 (3H, d, *J*=6.9 Hz, 21-H_3), 3.51 (1H, br s, 3-H), 3.55, 3.74 (1H each, both d, *J*=8.3 Hz, 19-H_2), 4.02 (1H, dd, *J*=2.2, 7.9 Hz, 22-H), 4.64 (1H, t-like, 23-H), 5.51 (1H, m, 24-H), 5.53 (1H, d, *J*=7.9 Hz, 1'-H), 5.62 (1H, dd, *J*=3.6, 9.9 Hz, 7-H), 6.14 (1H, d, *J*=9.9 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 633 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 635 $(M+H)^+$, 657 $(M+Na)^+$.

Karaviloside IX (**6**): a white powder, $[\alpha]_D^{26}$ –96.6° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{69}O_{14}$ $(M+H)^+$: 797.4688. Found: 797.4681. IR (KBr): 3453, 1082, 1036 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.74, 0.88, 0.91, 1.48, 1.63, 1.83 (3H each, all s, 18, 30, 29, 28, 26, 27-H_3), 1.14 (3H, d, *J*=6.9 Hz, 21-H_3), 3.58, 3.73 (1H each, both d, *J*=7.9 Hz, 19-H_2), 3.65 (1H, br s, 3-H), 4.01 (1H, dd-like, 22-H), 4.68 (1H, t-like, 23-H), 4.84 (1H, d, *J*=7.6 Hz, 1'-H), 5.54 (1H, d, *J*=7.9 Hz, 1''-H), 5.54

(2H, m, 7, 24-H), 6.19 (1H, d, *J*=9.6 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δc: given in Table 1. Negative-ion FAB-MS: *m/z* 795 $(M–H)^-$. Positive-ion FAB-MS: *m/z* 797 $(M+H)^+$, 819 $(M+Na)^+$.

Karaviloside X (**7**): a white powder, $[\alpha]_D^{28}$ –80.7° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{69}O_{14}$ $(M+H)^+$: 797.4687. Found: 797.4694. IR (KBr): 3432, 1084, 1034 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.75, 1.45, 1.63, 1.85 (3H each, all s, 18, 28, 26, 27-H_3), 0.89 (6H, s, 29, 30-H_3), 1.15 (3H, d, *J*=7.0 Hz, 21-H_3), 3.59, 3.76 (1H each, both d, *J*=8.3 Hz, 19-H_2), 3.62 (1H, br s, 3-H), 4.01 (1H, dd-like, 22-H), 4.63 (1H, t-like, 23-H), 5.34 (1H, d, *J*=7.9 Hz, 1'-H), 5.52 (1H, d, *J*=10.2 Hz, 24-H), 5.56 (1H, d, *J*=7.9 Hz, 1''-H), 5.77 (1H, dd, *J*=3.6, 9.8 Hz, 7-H), 6.18 (1H, d, *J*=9.8 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 795 $(M–H)^-$, 633 $(M–C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 797 $(M+H)^+$, 819 $(M+Na)^+$.

Karaviloside XI (**8**): a white powder, $[\alpha]_D^{27}$ –49.5° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{61}O_{10}$ $(M+H)^+$: 653.4265. Found: 653.4267. IR (KBr): 3432, 1084, 1032 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.79, 0.88, 0.89, 1.46, 1.61, 1.63 (3H, s, 18, 30, 29, 28, 26, 27-H_3), 1.12 (3H, d, *J*=6.4 Hz, 21-H_3), 3.58, 3.76 (1H each, both d, *J*=7.9 Hz, 19-H_2), 3.52 (1H, d-like, 24-H), 3.65 (1H, br s, 3-H), 4.53 (1H, ddd-like, 23-H), 5.37 (1H, d, *J*=7.9 Hz, 1'-H), 5.55 (1H, dd, *J*=3.9, 9.5 Hz, 7-H), 6.18 (1H, d, *J*=9.5 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 651 $(M–H)^-$. Positive-ion FAB-MS: *m/z* 653 $(M+H)^+$, 675 $(M+Na)^+$.

Acid Hydrolysis of Karavilosides (4–8): A solution of **4**–**8** (3 mg each) in 5% aqueous H_2SO_4–1,4-dioxane was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L–cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i) from **3**, **4** and **6**; D-allose (ii) from **5**, **6**, **7** and **8**; GLC conditions: column, Supeluco STB^{TM}-1, 30 m x 0.25 mm (i.d.) capillary column; column temperature, 230 °C; carrier gas, N_2; t_R, (i) 17.7 min (ii) 23.2 min (iii) 24.1 min (L-allose).

MeOH Treatment of karavilagenin C: A solution of karavilagenin C (5.0 mg, 0.01 mmol) in MeOH (2 mL) was refluxed for 48 hr. After cooling, the mixture was evaporated to dryness under reduced pressure and the residue was purified by reversed-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH (30 : 1, v/v)] to give karavilagenin A (1 mg, 19%). The obtained compound, karavilagenin A, was identified by comparison of EI-MS and ^{1}H-NMR data of isolated compound, karavilagenin A.

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