Momordica charantia Constituents and Antidiabetic Screening of the Isolated Major Compounds

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Bioguided fractionation of the methanol extract of *Momordica charantia* dried gourds led to the isolation of three new cucurbitane triterpenoids (1—3), together with eight known compounds (4—11). The aglycone of momordicoside I was isolated from the ether soluble fraction in a high amount. The structures of the metabolites were established on the basis of one and two dimensional NMR spectroscopic evidence, X-ray analysis, and comparison with the reported data in the literature. A number of phytochemicals have been isolated from *Momordica charantia* but the constituents responsible for the hypoglycaemic/antihyperglycaemic activities have not been determined. Therefore, in order to evaluate the contribution of the cucurbitane triterpenoids of the ether fraction of *M. charantia* methanol extract to *in vivo* anti-diabetic effects, the major compounds, 5β,19-epoxy-3β,25-dihydroxycucurbita-6,23(*E*)-diene (4), and 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (5) have been tested and have shown blood hypoglycaemic effects in the diabetes-induced male ddY mice strain at 400 mg/kg. The two aglycones of charantin did not show any hypoglycaemic effects. Our finding is the first demonstration that major pure cucurbutanoid compounds of *M. charantia* have *in vivo* hypoglycaemic effects.

Key words *Momordica charantia*; Cucurbitaceae; cucurbitane; antidiabetic, ddY mice

Diabetes mellitus is one of the most common chronic diseases and a major contributor to the development of cardiovascular diseases. It is due to a deficiency or a failure of normal action of insulin, which is responsible of the use of the sugar from the diet. The number of cases of non-insulin dependent diabetes mellitus has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity, and ageing of populations.[1] In the year 2000, the number of diabetic patients was 151 million and is estimated to rise to 300 million by 2025.[2,3]

The uses of natural drugs, such as plants and herbal remedies to treat diseases is very common in Asia and developing countries, where the population is linked with the use of traditional medicines, due to their efficiency or due the costs of the synthetic drugs and/or pharmaceuticals. One of the aims of phytochemists is to find the application of ethnomedicine in drug discovery. Moreover, WHO study groups emphasize strongly the optimal, rational uses of traditional and natural indigenous medicines (http://www.who.int/mediacentre/factsheets/fs134/en/). The leprosy gourd (bitter gourd, ayurveda name: karela), *Momordica charantia* L., a well known plant for its antidiabetic properties in Asia and some African countries, is among the candidates. Bitter and non-bitter cucurbitane triterpene aglycones and/or glycosides have been isolated from the plant.[4—11] The bitter principles so far reported have been characterized as momordicosides K and L, and momordicines I and II.[5,8] Interestingly, the four compounds have C-9 formyl, 7-OH or *O*-β-D-glucopyranosyl groups and are unsaturated at C-5, C-6. These features might be the structural requirement for the bitter taste and undoubtedly, the high content of saponins in the plant can be related to its taste.

The constituents responsible for the glucose lowering activity are not yet well known even though over hundred scientific articles have described the phytochemical and pharmacological properties of the plant.[12,13] In the course of our phytochemical screening of medicinal plants aimed at finding the active principles for antidiabetic activities, eleven compounds (**1**—**11**) were isolated from the Indian bitter gourd sample. The structural elucidation of compounds **1**—**3** is reported and the major compounds (**4** and **5**) have been tested against the antidiabetic strain of male ddY mice. The objective of the present study is to find the relationship between *Momordica charantia* constituents and the antidiabetic properties of the plant.

Results and Discussion

Effect of the Extracts on Diabetes Induced Mice The ether and ethyl acetate fractions of the water suspension of the bitter gourds methanol extract were tested for antidiabetic assay in mice. Oral administration of each fraction at 500 mg/kg (Figs. 3, 4, respectively) resulted marked hypoglycaemic effects comparable to glibenclamide (at 200 mg/kg).

Isolation and Characterization of Constituents from the Active Extracts A combination of size exclusion and silica gel column chromatography of the ether and ethyl acetate fractions of the methanol extract afforded eleven compounds (**1**—**11**). Compound **4** was previously obtained by hydrolysis of momordicoside I, but it has not been isolated from the *M. charantia* extract. The structure of compound **5** was determined to be 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien, 19-al, and confirmed by X-ray analysis (data not shown).[11] Compounds **6**—**9** were identified as: 5β,19-epoxycucurbita-6,23(*E*)-diene-3β,19,25-triol, 5β,19-epoxy-19-methoxycucurbita-6,23(*E*)-diene-3β,25-diol,[14] momordicoside L,[5] and *para*-methoxybenzoic acid, respectively, by comparison of their physical and spectroscopical data with those reported in the literature. Compounds **10** and **11** (isolated as mixture 1 : 1) were identified as sitosterol and stigmastadienol, which are the aglycones of charantin.

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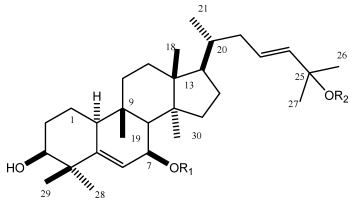
High resolution EI-MS of compound **1** supported a molecular formula of $C_{31}H_{52}O_3$. The ^{1}H-NMR spectrum of **1** exhibited signals for seven quaternary methyl groups at δ: 0.70, 0.92, 0.98, 1.04, 1.21 (s, each 3H), 1.31 (s, 6H), one secondary methyl at δ_H 0.88 (d, J=6.0 Hz), together with signals for two oxygen-bearing methines (δ_H 3.43, dd, 1H, J=5.4, 1.0 Hz and δ_H 3.51, br s), three olefinic protons (δ_H 5.57, d, J=15.8 Hz, H-23 and δ_H 5.59, m, H-6, H-24), and a signal for protons of a methoxyl group (δ_H 3.34, s, 7-OMe). The ^{13}C-NMR data displayed a resonance of a methoxyl group carbon together with 30 carbon signals assignable to a trihydroxycucurbita-5,23-diene. Moreover, the ^{13}C-NMR spectral data of **1** (Table 1) are very similar to those of 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (**5**)[11] except that the signal of the aldehyde group was absent and an additionnal resonance of a quarternary methyl group was observed (δ_H 0.98, s; δ_C 28.7). The placement of the methoxyl group at C-7 and the two hydroxyl groups at C-3 and C-25 were confirmed by COSY, HMBC, and NOESY experiments. The observation of the COSY correlations from H-6 to H-8 determined the assignment of an oxygen bearing methine to be at C-7 while the HMBC long-range correlations between H-3 and C-2, C-3, C-4, and C-5, and H-23 and C-24, and C-25 substantiated the two oxygen-bearing carbons at C-3 and C-25 (Fig. 1). The presence of cross peak between the methyl group at δ_H 0.98 and C-9, C-10, and C-8 in the HMBC spectrum substantiated the location of the additionnal methyl group to be at C-19. Moreover the methoxyl group must be located at C-7 and β-oriented, since the methoxyl protons at δ 3.34 showed long range correlation to the oxygen bearing carbon at δ_C 76.7, and a NOESY correlation between H-7 and CH_3-30. The orientation of the hydroxyl group at C-3 was substantiated by the coupling pattern of the signal due to H-3 (δ_H 3.51, br s). From the above data, the structure of **1** was deduced to be 3β,25-dihydroxy-7β-methoxycucurbita-5,23(*E*)-diene.

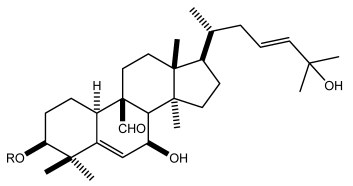
Table 1. ^{13}C-NMR Spectral Data for **1**—**3** and **5** (150 MHz)

	1[a]	**2**[a]	**3**[b]	**5**[b]
1	21.0	21.0	22.3	21.7
2	28.5	28.6	28.5 (−1.4)	29.9
3	77.2	77.2	87.0 (+11.4)	75.6
4	41.7	41.7	41.7	41.7
5	146.7	146.7	145.5	145.7
6	120.9	120.9	124.1	124.2
7	76.7	76.7	65.5	65.7
8	47.8	47.9	50.6	50.6
9	33.9	34.0	50.6	50.6
10	38.6	38.6	36.8	36.9
11	32.6	32.6	22.5	22.7
12	29.8	30.0	29.2	29.4
13	46.0	46.1	45.6	45.7
14	47.9	47.9	48.1	48.2
15	34.6	34.6	34.8	34.9
16	27.5	27.6	27.6	27.7
17	49.9	49.9	50.3	50.1
18	15.3	15.4	14.9	15.0
19	28.7	28.8	207.7	207.8
20	36.2	36.2	36.4	36.5
21	18.7	18.7	18.8	18.9
22	39.0	39.4	39.4	39.5
23	125.2	128.5	124.1	124.2
24	139.4	136.7 (−2.7)	141.6	141.7
25	70.7	74.8 (+4.1)	69.6	69.7
26	30.0	26.1 (−4.1)	30.7	30.8
27	29.9	25.8 (−3.9)	30.7	30.8
28	27.5	27.7	25.7	26.2
29	25.3	25.8	27.7	27.3
30	17.9	17.9	18.0	18.2
7-OCH_3	56.2	56.3		
25-OCH_3		50.2		
Allo-1			104.7	
2			73.4	
3			72.1	
4			69.0	
5			75.7	
6			63.2	

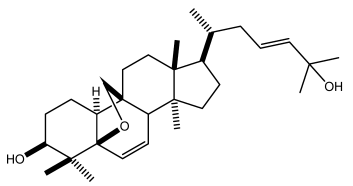
a) In $CDCl_3$; *b*) in C_5D_5N; values in parenthesis represented the upfield or downfield shifts.



1: R_1= Me, R_2= H
2: R_1= R_2= Me



3: R= β-D-Allopyranosyl
5: R=H



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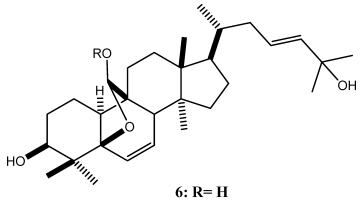
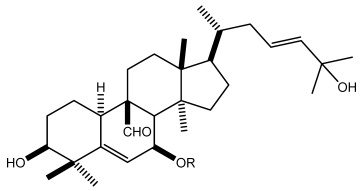
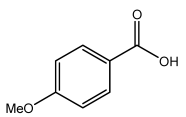


6: R= H
7: R= Me



8: Momordicoside L: R= β-D-Glucopyranosyl



9: *p*-Methoxybenzoic acid

Fig. 1. Chemical Constituents Isolated from *Momordica charantia*

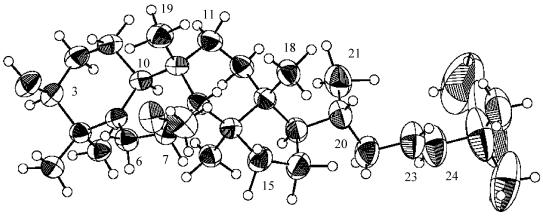


Fig. 2. The Ortep Drawing of **2**

Compound **2** exhibited a molecular formula of $C_{32}H_{54}O_3$ as determined by high resolution EI-MS. The ^{1}H- and ^{13}C-NMR data of **2** were very similar to those of **1** except the presence of signals due to a methoxyl group (δ_H 3.14, s; δ_C 50.2) in **2**. Comparison of the ^{13}C-NMR spectral data of **1** with those of **2** revealed the downfield shift of the carbon signal at C-25 (+4.1) and upfield shift of the signals at C-26 (−3.9), C-27 (−4.1), and C-24 (−2.7) of **1** (Table 1), indicating that the methoxyl group must be attached at C-25. Furthermore, HMBC experimental data showed the correlations between the methoxyl protons at δ_H 3.14 and δ_H 3.34, and the C-25 and C-7 resonances, respectively. In order to confirm the structure, its X-ray analysis was performed and the structure of **2** was confirmed to be 3β-hydroxy-7β,25-dimethoxycucurbita-5,23(*E*)-diene as depicted in Fig. 2.

The positive high resolution FAB-MS of compound **3** exhibited a quasimolecular ion peak due to $[M+K]^+$ at *m/z* 673.8391 ($C_{36}H_{58}O_9K$). The ^{1}H-NMR spectrum showed the presence of six quaternary methyl group singlet resonances at δ: 0.74, 0.84, 1.12, 1.55 (6H), 1.59, one secondary methyl group resonance at δ_H 0.96 (d, J=6.2 Hz), three olefinic proton resonances (δ_H 5.92, br m, H-23, 24 and δ_H 6.23, br d, J=5.4 Hz), and a signal of an anomeric proton at δ_H 5.34 (d, J=7.8 Hz, 1′-H) assignable to a β-allopyranosyl.

The ^{13}C-NMR spectrum exhibited 36 signals, 30 of which were assignable to 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (**5**), previously isolated from *M. charantia* collected from Nigeria,[11] and the six remaining to an allopyranosyl unit. On enzymatic hydrolysis by using hesperidinase, **3** gave **5** and D-allose as identified by GC/MS and by comparison of its optical rotation with that of authentic D-allose (see Experimental). Comparison of the ^{13}C-NMR spectral data of **3** and **5** revealed a downfield shift of C-3 (+11.4) and an upfield shift at C-2 (−1.4), indicating that C-3 position was the site of glycosylation. The location of the β-allopyranosyl group was confirmed by long range correlation between the anomeric proton resonance at δ 5.34 (d, J=7.8 Hz, 1′-H), and C-3 (δ 87.0). From the above data, the structure of **3** was confirmed as 3-*O*-β-D-allopyranosyl, 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al.

In order to prove if the methoxyl groups present in some of the isolated compounds are artifact produced during isolation or not, compounds **5** and **6** were dissolved in methanol and refluxed for 2 h. After evaporation of the solvent, each product (**5a**, **6a**) was freeze dried before the NMR measurement. In the ^{1}H-NMR of compound **5a**, a signal due to 7-OMe group (δ 3.44, s) appeared while C-25 remained hydroxylated. However, for compound **6a**, the presence of the two singlet signals at δ 3.13, and δ 3.42 indicated that both of the two hydroxyl groups at C-19 and C-25 were methylated. Thus, **2** and **7** were the artifacts of compound **1** and **6**,

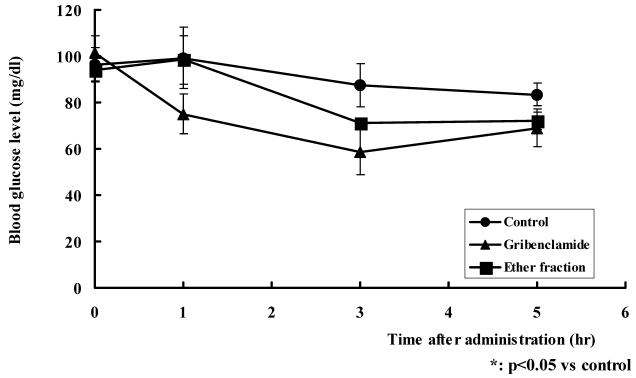


Fig. 3. Effect of the Ether Fraction in Blood Glucose Level in Diabetic Mice

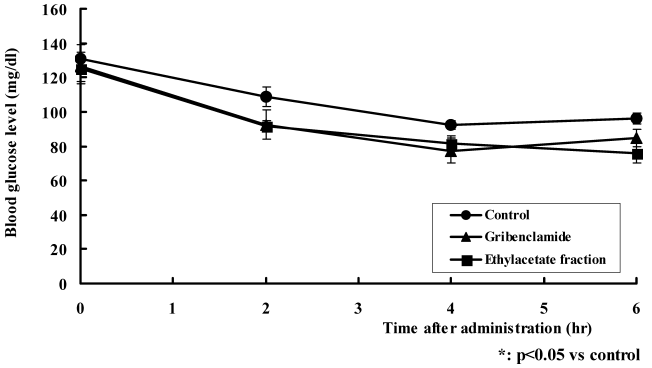


Fig. 4. Effect of the EtOAc Fraction in Blood Glucose Level in Diabetic Mice

respectively, and they can be produced during evaporation. However, 5β,19-epoxy-19-methoxycucurbita-6,23-diene-3β,25-diol and 5β,19-epoxy-19,25-dimethoxycucurbita-6,23-diene-3β-ol have been isolated from the $CHCl_3$ extract of *Momordica foetida*.[14] Noteworthy; this is the first isolation of cucurbitane triterpenoid having C-19 methyl, unsaturation at C-5 and C-6, C-7 hydroxyl and 2-methyl-hept-3-en-2-ol-6-yl side chain in nature.

Screening of the Antidiabetic Effect of the Isolated Major Constituents A mixture of steroidal saponins (charantin), insulin-like peptides and alkaloids have been reported to be the hypoglycaemic metabolites of *M. charantia*.[12] Moreover, apolypeptide-p, momordicin Ic, oleanolic acid 3-*O*-monodesmoside, and oleanolic acid 3-*O*-glucuronide have shown to possess hypoglycaemic activity.[13] The ether and the ethyl acetate fraction showed a promising results for the glucose lowering activity. Since compounds **4** and **5** were isolated in high amounts from the ether fraction, their *in vivo* antidiabetic activity was tested. Both two compounds significantly decreased the blood glucose levels as compared with those of control group (Fig. 5). For compound **4** significant differences in blood glucose level were observed at 2 and 6 h after administration as compared with those of control group, while compound **5** showed a significant decrease at 2 and 4 h with slight increase of the blood glucose level after 6 h. Although both compounds showed activity lower than glibenclamide (400 mg/kg), their high concentration in the extract and the presence of the previously reported bitter gourd constituents having hypoglycaemic activity could prove the antidiabetic properties of the plant.[12]

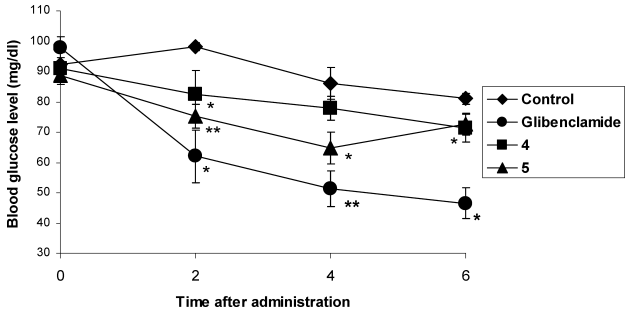


Fig. 5. Effect of Compounds **4** and **5** in Blood Glucose Level in Diabetic Mice

This study is the first demonstration that major pure cucurbutanoid constituents of *M. charantia* have *in vivo* hypoglycaemic effect. It is also interesting to note that compounds **10** and **11**, which are the aglycones of charantin,[12] did not show any marked effect on the blood glucose level (data not shown). *M. charantia* is a rich source of cucurbitane glycosides and the activity of EtOAc fraction on blood glucose might be due to glycosides. Unfortunately, the small amount of compounds isolated from the EtOAc extract did not allow us to make further investigation of their *in vivo* glucose lowering properties.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH or $CHCl_3$ as solvent. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 and/or 300 NMR spectrometer (600 or 300 MHz for ^{1}H and 150 or 75 MHz for ^{13}C), using either $CDCl_3$ or C_5D_5N (for glycosides) as solvent. Chemical shifts are given relative to tetramethylsilane (TMS, δ 0.00) as internal standard (^{1}H) and δ 77.02 from $CDCl_3$ and 123.5, 135.5, 149.5 (ppm) from C_5D_5N as standards (^{13}C). Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. X-Ray crystal analysis was carried out on a Mac Science DIP-2020. Column chromatography was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech, CH_2Cl_2–MeOH, 1 : 1) and silica gel (Kieselgel 60: 0.040—0.063 mm, Merck). The preparative HPLC experiment was performed using a Cosmosil reversed phase column, a JASCO 880-PU pump, JASCO 875-UV UV detector and ERC-7512 ERMA CR INC RI detector.

Plant Material The dried mature gourds of *M. charantia* L. were purchased at Sami Labs Ltd., Bangalore, India on April 2003. Voucher specimen was deposited in Sami Labs Ltd., 19/1, 19/2 I St. Main II Phase, Peenya Industrial Area, Peeya, Bengalore-560 058, India.

Extraction and Isolation Procedure Dried gourds of *M. charantia* (20 kg) were mechanically powdered and extracted with methanol at room temperature for one month. The extract was filtered and concentrated *in vacuo*. For preliminary screening, 415.8 g of the methanol extract was suspended in water, then extracted with ether, ethyl acetate and *n*-butanol successively to afford 61 g, 42 g, and 298 g of crude extracts, respectively. The ether extract (27 g) was subjected to silica gel column chromatography using a hexane–EtOAc gradient (by increasing the concentration of EtOAc) to divide it into twenty four fractions. Fraction 5 was applied to a Sephadex LH-20 column to afford three subfractions. A mixture of compounds **10** and **11** (53 mg) were obtained from HPLC on ODS RP-18 of the fraction 5-3. Fraction 9 was purified by Sephadex LH-20 to afford **9** (3.4 mg). Precipitation in EtOAc of the fraction 13 afforded compound **4** (178 mg). Fraction 17 yielded three subfractions by size exclusion chromatography. The fraction 17-2 was subjected to CC on silica gel (Hexane : EtOAc : Et_2O=4 : 3 : 1), affording compound **5** (175 mg). Fraction 20 was divided into three fractions by Sephadex LH-20. Further purification of the fraction 20-2 was performed on silica gel column (from Hexane : EtOAc=6 : 4 to 100% EtOAc) to give **6** (25.2 mg), **1** (3.2 mg), **5** (76 mg), and **7** (21.3 mg).

The ethyl acetate fraction (3 g) was subjected to a silica gel column chromatography (CH_2Cl_2 : MeOH : H_2O=15 : 3 : 1-lower phase, and EtOAc) to afford eight fractions. Compound **2** (9.3 mg) was obtained from fraction 1 by a combination of silica gel column chromatography and RP-18 ODS-HPLC (*n*-hexane : EtOAc=7 : 3 to 100% EtOAc, and 95% MeOH, respectively), while purification of fraction 3 by Sephadex LH-20 column and ODS RP-18 (CH_3CN : H_2O=13 : 7) gave **3** (7.8 mg).

The *n*-butanol fraction was subjected to DIAION column and eluted by H_2O and MeOH by increasing the MeOH content (H_2O, 50% MeOH, MeOH and then Acetone). Combination of silica gel (using CH_2Cl_2 : MeOH : H_2O=15 : 3 : 1-lower phase; CH_2Cl_2 : MeOH=9 : 1, 4 : 1), ODS RP-18 (MeOH : H_2O=9 : 1) and silica gel chromatography ($CHCl_3$: MeOH=17 : 3) of 16.2 g of the fraction eluted with MeOH afforded compound **8** (8.4 mg).

3β,25-Dihydroxy-7β-methoxycucurbita-5,23(*E*)-diene (**1**): Amorphous powder, $[\alpha]_D^{20}$ −73.2° (*c*=0.8, $CHCl_3$), IR (KBr): 3336, 1449, 1068 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.70, 0.92, 0.98, 1.04, 1.21 (each s and 3H, 30, 18, 19, 29, 28-CH_3), 0.88 (d, *J*=6.0 Hz, 3H, 21-CH_3), 1.31 (s, 6H, 26-, 27-CH_3), 2.16 (m, H-22), 2.28 (dd, 1H, *J*=12.5, 4.5 Hz, H-10), 3.34 (s, 7-OMe), 3.43 (dd, 1H, *J*=5.4, 1.0 Hz, 7-H), 3.51 (br s, H-3), 5.57 (d, *J*=15.8 Hz, H-23), 5.59 (m, H-6, H-24). ^{13}C-NMR see Table 1. Positive HR-EI-MS *m/z* 472.3915 $[M]^+$, $C_{31}H_{52}O_3$, requires 472.3905.

3β-Hydroxy-7,25-dimethoxycucurbita-5,23(*E*)-diene (**2**): Amorphous powder, $[\alpha]_D^{17}$ −15.3° (*c*=1.5, $CHCl_3$), IR (KBr): 3453, 2943, 1467, 1032 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.69, 0.92, 1.03, 1.20 (each s and 3H, 30-, 18, 19-, 28-CH_3), 1.24 (s, 6H, 26-, 27-CH_3), 0.98 (s, 3H, H-29), 0.89 (d, *J*=6.0 Hz, 3H, 21-CH_3), 2.18 (m, H-22), 2.27 (dd, 1H, *J*=12.5, 4.5 Hz, H-10), 3.14 (s, 25-OMe), 3.34 (s, 7-OMe), 3.42 (br d, 1H, *J*=5.4 Hz, 7-H), 3.51 (br s, H-3), 5.37 (d, *J*=15.8 Hz, H-23), 5.50 (ddd, *J*=15.8, 8.7, 5.7 Hz, H-24), 5.83 (d, *J*=5.4 Hz, H-6). ^{13}C-NMR see Table 1. Positive HR-EI-MS *m/z* 486.4051 $[M]^+$, $C_{32}H_{54}O_3$, requires 486.4073.

X-Ray Crystallographic Analysis of 2 Crystal data: $C_{32}H_{54}O_3$, Mr=486.781, Orthorhombic, $P2_12_12_1$, *a*=7.7220(4) Å, *b*=10.0960(7) Å, *c*=38.762(4) Å, α=90.00°, β=90.00°, γ=90.00°, *V*=3021.9(4) Å^3, Mo$K\alpha$ radiation, λ=0.71073, 4333 reflections, 317 parameters; only coordinates of H atoms refined, *R*(gt)=0.0738, w*R*(gt)=0.2088, *S*(ref)=1.043. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL), data reduction: maXus.[15] Program used to refine structure: *SHELXL*-97[16]; Refinement on F^2, full matrix least squares calculations.

3-*O*-β-D-Allopyranosyl-7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al (**3**): $[\alpha]_D^{20}$ +11.3° (*c*=0.9, MeOH), IR (KBr): 3378, 2949, 1464, 1081 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 0.81, 0.91, 1.08 (each s and 3H, 30-, 18-, 29-CH_3), 0.94 (d, *J*=6.0 Hz, 3H, 21-CH_3), 1.25 (s, 6H, 26-, 27-CH_3), 1.31 (3H, s, 28-CH_3), 3.27 (dd, *J*=7.9, 3.2 Hz, 1H, H-7), 3.44 (dd, *J*=9.4, 2.1 Hz, 1H, H-4′), 3.63 (m, 2H, 6a′-H, 5′-H), 3.79 (d, *J*=9.2 Hz, 1H, 6b′-H), 3.99 (br s, 1H, 3-H), 4.02 (d, *J*=6.0 Hz, 2′-H), 4.60 (d, *J*=7.6 Hz, 1′-H), 5.57 (m, H-23), 5.87 (br d, *J*=5.4 Hz, 6-H), 10.23 (s, 1H, 19-H). (300 MHz, C_5D_5N) δ: 0.74, 0.84, 1.12, (each s and 3H, 30-, 18-, 29-CH_3), 0.96 (d, *J*=6.2 Hz, 21-CH_3), 1.55 (s, 6H, 26-, 27-CH_3), 1.59 (s, 3H, 28-CH_3), 3.87 (dd, *J*=7.9, 3.2 Hz, 1H, H-7), 3.95 (br s, 1H, H-3), 4.18 (dd, *J*=9.4, 2.1 Hz, 1H, H-4′), 4.35 and 4.49 (m, 2H, 6a′-H, 5′-H), 5.34 (d, *J*=7.8 Hz, 1′-H), 5.92 (br m, H-23, 24), 6.23 (br d, *J*=5.4 Hz, 6-H), 10.60 (s, 19-H). ^{13}C-NMR see Table 1. Positive HR-FAB-MS *m/z* 673.8391 $[M+K]^+$, $C_{36}H_{58}O_9K$, requires 673.8405.

Enzymatic Hydrolysis of Compound 3 An acqueous solution of **3** (10 mg) and hesperidinase (5 mg) was incubated at 37 °C for 3 d. The solution was extracted with EtOAc and the produced aglycone (6.3 mg) was identified as 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien, 19-al (**5**) by comparison of its spectral data with those reported in the reported in the literature (Fatope *et al.*, 1990). The acqueous layer was freeze-dryed to afford D-allose (2.1 mg) as identified by its optical rotation ($[\alpha]_D^{17}$ +9.2°, *c*=1.8, H_2O) and comparison of the GC/MS of its TMS derivative with that of authentic D-allose [TMS derivative of sample or authentic D-allose was prepared by adding 1 ml CH_3CN and 250 μl *N*,*O*-bis(trimethylsilyl)-acetamide in 1 mg of sample or authentic D-allose. The mixture was allowed to stand for 30 min before GC/MS analysis].

Methanolysis of Compounds 5 and 6 Each compound (3 mg) was dissolved in MeOH (3 ml) and refluxed for 2 h. After evaporation of the solvent, each product (**5a**, **6a**) was freeze dried for 4 h before NMR measurement. **5a**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88, 0.90, 1.04 (each s, 3×CH_3), 0.99 (d, *J*=6.0 Hz, 3H, CH_3), 1.23 (s, 6H, 2×CH_3), 1.30 (s, 3H, CH_3), 3.44 (s, 3H, 7-OMe), 3.97 (br s, 1H, 3-H), 4.42 (br d, 1H, *J*=5.5 Hz, 7-H), 5.59 (m, H-23, 24), 5.90 (br d, *J*=5.4 Hz, 6-H), 9.73 (s, 1H). **6a**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.83, 0.84, 0.86 and 0.88 (each s, 3H, 4×CH_3), 1.20 (d, *J*=6.0 Hz, 3H, CH_3), 1.25 (s, 6H, 2×CH_3), 3.13 (s, 3H, 19-OMe), 3.39 (br s, 1H, 3-H), 3.42 (s, 3H, 25-OMe), 4.63 (s, 1H, 19-H), 5.38 (d, *J*=15.9 Hz, H-24), 5.48 (m, H-23), 5.50 (m, 7-H), 6.05 (dd, *J*=10.0, 2.5 Hz,

6-H).

Evaluation of Antidiabetic Activity. Animals Male ddY strain mice weighing about 30 g were purchased from SLC (Shizuoka) and housed for 1 week prior to the commencement of the experiments under a constant temperature of 21±2 °C, humidity of 55±15%, and a 12 h light/dark cycle.

Hypoglycaemic Activity Diabetes was induced by the intravenous administration of alloxan (Sigma) at a dose of 40 mg/kg. Five days after diabetes induction, mice were fasted for 18 h. Each test sample was suspended in 1% gum arabic solution. Glibenclamide (Sigma) was used as positive control at a dose 200 mg/kg or 400 mg/kg. Blood glucose levels were measured with Glucometer DEX (Bayer Health Care, U.S.A.) at 1, 3 and 5 h or 2, 4 and 6 h after sample administration. In the control mice, 1% gum arabic solution was administered instead of sample. If test samples were difficult to suspend in 1% gum arabic solution, additional gum arabic powder was added at appropriate amount. The addition of gum arabic to 1% solution is confirmed not to affect on the blood glucose level in case of that the solution was administered to mice. Each sample was tested by using 3—5 mice.

On the evaluation of the bioactivity of **4** and **5**, a dose of 200 mg/kg as gribenclamide was used. Pre-values of blood glucose level of control group, gribenclamide-treated group, compound **4**-treated group and compound **5**-treated group were 92.4, 98.0, 91.0 and 88.6 mg/dl, respectively. There was statistically no difference between the four groups.

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