The Cucurbitaceae plant *Momordica* (*M.* charantia) L. (Japanese name “Tsurureishi”) is cultivated as a vegetable in Asian countries. In Chinese, Indian Ayurvedic, and Indonesian Jamu traditional medicines, the fruit of this plant has been used as a bitter stomachic, a laxative, an antidiabetic, and an anthelmintic for children. Recently, the alcoholic extract from the fruit of *M. charantia* originated in Sri Lanka was reported to inhibit the increase of serum glucose in glucose-loaded rats. As chemical constituents of the fruit, many cucurbitane-type triterpene glycosides were reported, but the constituents responsible for the inhibition of glucose absorption have not been identified. In the course of our studies on the bioactive constituents of medicinal foodstuffs, we have also characterized many triterpene glycosides from the fruit of *M. charantia* originated in Japan, Thailand, Indonesia, India, and Sri Lanka. In this paper, we present the isolation and structure elucidation of goyaglycosides-a (1), -b (2), -c (3), -d (4), -e (5), -f (6), -g (7), and -h (8) and goyasaponins I (9), II (10), and III (11) from the fresh fruit of Japanese *M. charantia*, which is commonly called “nigauri” or “goya”.

The cucurbitane- and oleanene-type triterpene glycosides of the fresh fruit of Japanese *M. charantia* were separated by the procedures shown in Chart 1. The methanolic extract obtained from the fresh fruit of *M. charantia* was partitioned into an ethyl acetate and a water mixture to give an ethyl acetate extract and an aqueous phase. The aqueous phase was extracted with 1-butanol to furnish a 1-butanol extract and an aqueous extract. The 1-butanol extract was subjected to normal- and reversed-phase silica gel column chromatography and finally HPLC to give goyaglycosides-a (1), -b (2), -c (3), -d (4), -e (5), -f (6), -g (7), and -h (8), goyasaponins I (9), II (10), and III (11), and momordicosides A3a (12), C3b (13), F13c (14), I3c (15), and K3d (16).

**Structures of Cucurbitane-Type Triterpene Glycosides (1—8)** Goyaglycoside-a (1) was obtained as a white powder.

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**Key words** *Momordica charantia*; goyaglycoside; goyasaponin; nigauri; cucurbitane-type triterpene glycoside; oleanane-type triterpene saponin


der with negative optical rotation ([α]_{D}^{26} = -101.4^\circ). The IR spectrum of \( \mathbf{1} \) showed strong absorption bands at 3423 and 1080 cm\(^{-1}\) suggestive of a glycosidic function. In the negative- and positive-ion FAB-MS of \( \mathbf{1} \), quasimolecular ion peaks were observed at \( m/z 647 \) (M–H)\(^+\) and \( m/z 649 \) (M+H)\(^+\) and the molecular formula \( \text{C}_{37}\text{H}_{60}\text{O}_{9} \) was determined by high-resolution MS measurement. Furthermore, fragment ion peaks of \( \mathbf{1} \) were observed at \( m/z 617 \) (M–CH\(_3\)O)\(^+\) and \( m/z 455 \) (M–CH\(_3\)O–C\(_{6}\)H\(_{10}\)O\(_{5}\))\(^+\). Acid hydrolysis of \( \mathbf{1} \) with 5% aqueous sulfuric acid (H\(_2\)SO\(_4\))–1,4-dioxane (1 : 1, v/v) furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.\(^5\) While enzymatic hydrolysis of \( \mathbf{1} \) with naringinase furnished 19(R)-methoxy-5β,19-epoxycucurbita-6,23-diene-3β,25-diol (\( \mathbf{17} \)).\(^6\) The \(^1\)H-NMR and \(^13\)C-NMR spectra of \( \mathbf{1} \), which were assigned by various NMR experiments,\(^7\) showed signals due to the aglycone moiety \( [\delta 0.86, 0.89, 0.90, 1.47 \text{ (all s, 29, 30, 18, 28-H\(_3\))}, 0.97 \text{ (d, } J=5.3 \text{ Hz, 21-H\(_3\))}, 1.54 \text{ (s, 26, 27-H\(_3\))}, 3.12 \text{ (dd-like, 8-H)}), 3.44 \text{ (s, 19-OMe)}, 3.73 \text{ (br s, 3-H)}, 4.84 \text{ (s, 19-H)}, 5.62 \text{ (dd, } J=3.6, 9.6 \text{ Hz, 7-H)}), 5.92 \text{ (m, 23, 24-H)}, 6.16 \text{ (dd, } J=2.0, 9.6 \text{ Hz, 6-H})] \text{ and a } \beta\text{-D-glucopyranosyl moiety} \[ \delta 4.95 \text{ (d, } J=7.6 \text{ Hz, 1'-H})]. \text{ The bonding position of the } \beta\text{-D-glucopyranosyl moiety in } \mathbf{1} \text{ was clarified by HMBC experiment, which showed a long-range correlation between the 1'-proton of the glucopyranosyl moiety and the 3-carbon of the aglycone moiety. Consequently, the structure of goyaglycoside-a was determined to be 19(R)-methoxy-5β,19-epoxycucurbita-6,23-diene-3β,25-diol 3-O-β-D-glucopyranoside (\( \mathbf{1} \)).}\(^8\)

Goyaglycoside-b (\( \mathbf{2} \)), obtained as a white powder with negative optical rotation, liberated d-allose by acid hydrolysis, which was also identified by GLC analysis of the thiazolidine derivative.\(^5\) The molecular formula \( \text{C}_{37}\text{H}_{60}\text{O}_{9} \) of \( \mathbf{2} \) was determined from quasimolecular ion peaks \( [m/z 647 \text{ (M–H)}\(^−\) \text{ and 649 (M+H)}\(^+\)] \) in the negative- and positive-ion FAB-MS and
by high-resolution MS measurement. The $^1$H-NMR (pyr-
dine-$d_5$) and $^{13}$C-NMR (Table 1) spectra$^7$ of 2 showed sig-
nals due to a 19(R)-methoxy-5β,19-epoxycucurbita-6,23-
diene-3β,25-diol moiety [δ 0.83, 0.89, 0.90, 1.44 (all s, 29,
30, 18, 28-H$_3$), 0.98 (d, $J=5.3$ Hz, 21-H$_3$), 1.54 (s, 26, 27-
H$_3$), 3.13 (dd-like, 8-H), 3.50 (s, 19-OMe), 3.70 (br s, 3-H),
4.88 (s, 19-H), 5.61 (dd, $J=3.3$, 9.6 Hz, 7-H), 5.92 (m, 23,
24-H), 6.15 (dd, $J=2.0$, 9.6 Hz, 6-H)] together with an β-D-
allopyranosyl moiety [δ 5.44 (d, $J=7.6$ Hz, 1′-H)]. In the
HMBC experiment of 2, a long-range correlation was ob-
served between the 1′-proton of the allopyranosyl moiety and
the 3-carbon of the aglycone. Finally, comparison of the
NMR data for 2 with those for 1 led us to formulate the
structure of goyaglycoside-b as 19(R)-methoxy-5β,19-epoxycucurbita-6,23-
diene-3β,25-diol 3-β-D-allopyranoside (2).$^3$

Goyagylicosides-c (3) and -d (4), obtained as a white pow-
der with negative optical rotation, were found to have the same molecular formula, C$_{38}$H$_{60}$O$_{15}$, which was determined from the quasimolecular ion peaks [m/z 661 (M − H$^-$), m/z 685 (M + Na$^+$)] in the negative- and positive-ion FAB-MS
and by high-resolution MS measurement. Acid hydrolysis of 3
furnished α-glucose, while β-allose was obtained by acid
hydrolysis of 4.$^1$ Enzymatic hydrolysis of 3 with naringinase
furnished 19(R),25-dimethoxy-5β,19-epoxycucurbita-6,23-
diene-3β,25-diol 3-β-D-allopyranoside (4).$^3$

The carbon and proton signals in the $^1$H-NMR (pyr-
dine-$d_5$) and $^{13}$C-NMR (Table 1) spectra$^7$ of 3 and 4 were super-
imposable on those of 1 and 2, except for signals due to the
25-methoxyl group, respectively. The $^1$H-NMR spectra of 3
and 4 showed two methoxyl signals to the 19- [3: δ 3.44 (s);
4: δ 3.50 (s)] and 25-positions [3, 4: δ 3.22 (s)]. Furthermore,
the HMBC experiments on 3 and 4, long-range corre-
lations were observed between the 25-methoxyl protons
and the 25-carbon and between the 1′-proton [3: δ 4.95 (d,
$J=7.6$ Hz); 4: δ 5.43 (d, $J=7.7$ Hz)] and the 3-carbon. Con-
sequently, the structures of goyaglycosides-c and -d were
characterized as 19(R),25-dimethoxy-5β,19-epoxycucurbita-6,23-
diene-3β,25-diol 3-β-D-glucopyranosyl moiety [δ 2.0, 9.6 Hz, 6-H]
and positive-ion FAB-MS and by high-resolution MS measure-
ment. Furthermore, a fragment ion peak was observed at m/z 617 (M − C$_6$H$_{11}$O$_5$)$^-$ in the negative-ion FAB-MS of 5. Acid hydrolysis of 5 with 5%
apaque H$_2$SO$_4$–1,4-dioxane (1:1, v/v) furnished β-allose and α-glucose.$^5$ Enzymatic hydrolysis of 5 with naringinase
furnished momordicoside F$_2$ (19).$^5$ The $^1$H-NMR (pyr-
dine-$d_5$) and $^{13}$C-NMR (Table 1) spectra$^7$ of 5 showed signals as-
signed to a β-D-glucopyranosyl moiety [δ 4.98 (d, $J=
7.6$ Hz, 1′-H)] together with a momordicoside F$_2$ moiety [δ
0.98 (d, $J=5.6$ Hz, 21-H$_3$), 2.31 (brs, 8-H), 3.64 (brs, 3-H),
3.65, 3.71 (ABQ, $J=7.9$ Hz, 19-H$_3$), 5.35 (d, $J=8.5$ Hz, 1′-
H$_3$), 5.56 (dd, $J=3.3$, 9.9 Hz, 7-H), 5.79 (ddd-like, 23-H),
6.01 (d, $J=15.8$ Hz, 24-H), 6.18 (d, $J=9.9$ Hz, 6-H)] in the
HMBC experiment of 5, long-range correlations were ob-
served between the 1′-proton of the β-allopyranosyl moiety
and the 3-carbon and between the 1′-proton of the β-glu-
copyranosyl moiety and the 25-carbon. Consequently, the structure of goyaglycoside-e was determined as 25-O-β-D-glucopyranosyl-5β,19-epoxyecurbita-6,23-diene-3β,25-diol 3-O-β-D-allopyranoside (5).

Goyaglycoside-f (6) was isolated as a white powder with negative optical rotation ([α]D 20 = −51.9%). In the negative- and positive-ion FAB-MS of 6, quasimolecular ion peaks were observed at m/z 779 (M−H)− and m/z 781 (M+H)+ and the molecular formula C42H68O13 was determined by high-resolution MS measurement. Acid hydrolysis of 6 with 5% aqueous H2SO4-1,4-dioxane (1:1, v/v) furnished D-allose and D-lopyranosyl moiety.

The molecular formula C42H68O13 was determined by high-resolution MS measurement. Negative-ion FAB-MS of 5 and 6, and the 23-C (Fig. 1). The stereostructure of the aglycone moiety was determined to be 23-O-Me by NOESY experiment, which served at

Table 1. 13C-NMR Data for Goyaglycosides-a (1), -b (2), -c (3), -d (4), -e (5), -f (6), -g (7), and -h (8) and Goyagenin A (20)

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The spectra were taken in pyridine-d5 at 68 MHz.
Goyaglycoside-g (7), obtained as a white powder, liberated D-allose and D-glucose by acid hydrolysis. The molecular formula $C_{42}H_{70}O_{15}$ of 7 was obtained from quasimolecular ion peaks $m/z$ 809 (M−H) and 833 (M+Na)$^+$ in the negative and positive-ion FAB-MS. Enzymatic hydrolysis of 7 with cellulase furnished goyaglycoside-b (2). The $^1$H-NMR (pyridine-d$_6$) and $^{13}$C-NMR (Table 1) spectra$^7$ of 7 showed signals due to a goyaglycoside-b moiety [δ 0.83, 0.91, 0.92, 1.44, 1.54, 1.60 (all s, 29, 18, 30, 28, 26, 27-H$_3$), 1.00 (d, $J$ = 5.3 Hz, 21-H$_3$), 3.12 (dd-like, 23-H), 3.50 (s, 19-OMe), 3.69 (br s, 3-H), 4.89 (s, 19-H), 5.43 (d, $J$ = 7.6 Hz, 1’-H), 5.61 (dd, $J$ = 3.3, 9.9 Hz, 7-H), 5.79 (ddd, $J$ = 5.6, 7.9, 15.8 Hz, 23-H), 6.01 (d, $J$ = 15.8 Hz, 24-H), 6.15 (dd, $J$ = 2.3, 9.9 Hz, 6-H)] together with a β-D-glucopyranosyl moiety [δ 4.99 (1H, d, $J$ = 7.9 Hz, 1’-H)]. In the HMBC experiment of 7, a long-range correlation was observed between the 1’-proton of the glucopyranosyl moiety and the 25-carbon. Consequently, the structure of goyaglycoside-g was determined to be 25-β-D-glucopyranosyl-19(R)-methoxy-5β,19-epoxyxycucurbita-6,23-diene-3β,25-diol 3-O-β-D-glucopyranoside (7).$^8$

Goyaglycoside-h (8) was isolated as a white powder with positive optical rotation ([α]$_{D}^{20}$ + 0.7°). In the negative- and positive-ion FAB-MS of 8, quasimolecular ion peaks were observed at $m/z$ 813 (M−H)$^-$ and $m/z$ 837 (M+Na)$^+$. High-resolution MS analysis revealed the molecular formula of 8 to be $C_{42}H_{70}O_{15}$. By acid hydrolysis with 5% aqueous H$_2$SO$_4$–1,4-dioxane (1 : 1, v/v), 8 liberated D-glucose.$^5$ Enzymatic hydrolysis of 8 with naringinase furnished a new triterpene aglycone called goyaglycosin A (20), whose molecular formula $C_{30}H_{50}O_{5}$ was determined from the positive-ion FAB-MS $m/z$ 513 (M+Na)$^+$ and by high-resolution MS measurement. The IR spectrum of 20 showed absorption bands at 3567 and 1717 cm$^{-1}$ suggestive of hydroxyl and carbonyl functions. The $^1$H-NMR (pyridine-d$_6$) and $^{13}$C-NMR (Table 1) spectra$^7$ of 8 showed signals due to six tertiary methyls [δ 0.80, 0.88, 0.91, 1.06, 1.48, 1.63, 1.72 (all s, 30, 19, 28, 18, 29, 27, 26-H$_3$)], three methines bearing a hydroxyl group [δ 3.73 (brs, 3-H), 4.81 (dd-like, 22-H), 5.53 (d-like, 23-H)], a secondary methyl [δ 1.50 (d, $J$ = 6.3 Hz, 21-H$_3$)], a olefin [δ 5.45 (dd-like, 6-H)], and two β-D-glucopyranosyl moieties [δ 4.80 (d, $J$ = 7.6 Hz, 1’-H)], 5.17 (d, $J$ = 7.6 Hz, 1’-H)] together with a carbonyl and four quaternary carbons. The proton and carbon signals of 8 in $^1$H- and $^{13}$C-NMR spectra were superimposable on those of a known cucurbitane-type triterpene saponin, momordicoside A (12),$^{13b}$ except for those around the 24-position. In the HMBC spectra of 8, long-range correlations were observed between the following protons and carbons: Glc-1’-H and 3-C; Glc-6’-C and Glc-6’-C; 26-, 27-H$_3$ and 24-C. These findings and comparisons of the $^1$H- and $^{13}$C-NMR spectra of 8 with those of known momordicosides$^3$ led us to formulate the structure of 8 as 3,22,23,25-tetrahydroxycucurbit-5-ene-24-one 3-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

In order to learn the absolute stereostructure of 8, the aglycone, goyaglycosin A (20) was treated with sodium borohydride (NaBH$_4$) to furnish 3β,22(S),23(R),24(R),25-pentahydroxycucurbit-5-ene (21)$^{15a}$ and its 24-diastereomar (22) as shown in Fig. 2. On the basis of the evidence, goyaglycoside-h was determined to be 3β,22(S),23(R),25-tetrahydroxycucurbit-5-en-24-one 3-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.
aqueous H$_2$SO$_4$–1,4-dioxane (1 : 1, v/v) furnished D-xylose, while methanolysis of L-rhamnose, D-fucose, D-galactose, and D-glucuronic acid, 5) indicated the presence of a glycogen moiety [δ 5.18 (d, J = 6.1 Hz, Fuc-6-H$_3$), 6.27 (d, J = 5.5 Hz, Fuc-5-H$_2$)] while methanolysis of 9 with 9% HCl-dry methanol liberated gypsogenin (23).10 Alkaline hydrolysis of 9 with 5% aqueous NaOH provided gypsosaponin (24).

By acid hydrolysis with 5% aqueous H$_2$SO$_4$–1,4-dioxane (1 : 1, v/v), goyasospondin (24) liberated d-glucuronic acid and d-galactose.5) The molecular formula C$_{65}$H$_{102}$O$_{31}$ of 24 was also determined from the negative- and positive-ion FAB-MS [m/z 1231 (M–C$_6$H$_6$O$_6$)$_{-}$], 1215 (M–C$_6$H$_5$O$_3$)$_{-}$, 1099 (M–C$_5$H$_5$O$_3$)$_{-}$, 1039 (M–C$_4$H$_4$O$_2$)$_{-}$, and 807 (M–C$_3$H$_3$O$_2$)$_{-}$] in the negative-ion FAB-MS of 9. Acid hydrolysis of 9 with 5% aqueous H$_2$SO$_4$–1,4-dioxane (1 : 1, v/v) furnished D-xyllose, L-rhamnose, D-fucose, D-galactose, and D-glucuronic acid.5) The IR spectrum of 9 showed absorption bands at 3410, 1717, 1684, 1078, and 9930 cm$^{-1}$ due to hydroxyl, carbonyl, and carbonyl functions. In the negative- and positive-ion FAB-MS of 9, quasimolecular ion peaks were observed at m/z 1377 (M–H$^+$) and m/z 1401 (M+Na$^+$) and the molecular formula C$_{65}$H$_{102}$O$_{31}$ was determined by high-resolution MS measurement. Furthermore, fragment ion peaks were observed at m/z 1231 (M–C$_6$H$_6$O$_6$)$_{-}$, 1215 (M–C$_6$H$_5$O$_3$)$_{-}$, 1099 (M–C$_5$H$_5$O$_3$)$_{-}$, 1039 (M–C$_4$H$_4$O$_2$)$_{-}$, and 807 (M–C$_3$H$_3$O$_2$)$_{-}$] in the negative-ion FAB-MS of 9. Acid hydrolysis of 9 with 5% aqueous H$_2$SO$_4$–1,4-dioxane (1 : 1, v/v) furnished D-xyllose, L-rhamnose, D-fucose, D-galactose, and D-glucuronic acid.5) The molecular formula C$_{65}$H$_{102}$O$_{31}$ of 24 was also determined from the negative- and positive-ion FAB-MS [m/z 807 (M–H$^+$), m/z 831 (M+Na$^+$)] and by high-resolution MS measurement. The $^1$H-NMR (pyridine-d$_5$) and $^{13}$C-NMR (Table 2) spectra$^7$ of 24 showed signals due to the glycosidic moieties [δ 83.26 (dd-like, 18-H), 4.06 (dd-like, 3-H), 5.43 (br s, 12-H), 9.92 (s, 23-H)] and the disaccharide moieties consisted of a β-d-glucopyranosiduronic acid [δ 4.90 (d, J = 7.3 Hz, GlcA-1-H)] and a β-d-galactopyranosyl [δ 5.18 (d, J = 7.6 Hz, Gal-1-H)] part. The disaccharide structure bonding to the 3-position of 24 was determined by HMBC experiment. Thus, long-range correlations were observed between the 1-proton of the glucuronic acid part and the 3-carbon of the glycosidic part and between the 1-proton of the galactopyranosyl moiety and the 2-carbon of the glucuronic acid part. Based on this evidence, the structure of goyasosaponin (24) was elucidated.

The spectra were taken in pyridine-d$_5$ at 125 MHz.
Namely, long-range correlations were observed between the following protons and carbons: [C6H11O4] (M/z 1509 (M−H)−) and [C6H11O5] (M/z 1533 (M+Na)+) ion FAB-MS and by high-resolution MS measurement. Furthermore, fragment ion peaks were observed at [C6H11O4]− 1363 (M−C6H11O3)−, 1347 (M−C6H11O2)−, 1245 (M−C10H16O5)−, 1171 (M−C12H10O5)−, and 1099 (M−C9H5O3)− observed in the negative-ion FAB-MS of 10. Acid hydrolysis of 10 with 5% aqueous H2SO4–1,4-dioxane (1:1, v/v) furnished d-xyllose, d-fucose, d-galactose, and d-glucuronic acid. The high-resolution and 13C-NMR (Table 2) spectra of 10 showed signals assignable to a β-D-glucopyranosiduronic acid moiety [δ 4.89 (d, J = 7.3 Hz, GlcA-1-H)], a β-D-galactopyranosyl moiety [δ 5.20 (d, J = 7.3, Gal-1-H)], a β-D-fucopyranosyl moiety [δ 5.15 (d, J = 5.8 Hz, Fuc-6-H)], 6.28 (d, J = 5.5 Hz, Fuc-1-H)], two α-L-rhamnopyranosyl moieties [δ 1.61 (d, J = 6.1 Hz, Rha-6′-H)], 1.68 (d, J = 5.5 Hz, Rha-6′-H), 5.65 (brs, Rha-1′-H), 5.73 (brs, Rha-1-H), two β-D-xylpyranosyl moieties [δ 5.07 (d, J = 7.0 Hz, Xyl-1-H)], 5.18 (d, J = 7.3 Hz, Xyl-1′-H), and a glycosin moiety [δ 3.11 (dd-like, 18-H), 4.04 (dd, J = 5.8, 15.0 Hz, 3-H), 5.44 (brs, 12-H), 9.93 (s, 23-H)]. The oligosaccharide structure of 10 was characterized by a HMBC experiment, which showed long-range correlations between the following protons and carbons: GlcA-1-H and 3-C, Gal-1-H and Galc-1-C, and Glc-1-H and GlcA-4-C; GlcA-3-H and Ac-1-C.

Goyasaponin I (II), isolated as a white powder, furnished goyasaprosapogenin (24) upon alkaline hydrolysis. The molecular formula C24H36O12 was determined by the negative- and positive-ion FAB-MS [quasimolecular ion peaks: m/z 949 (M−H)−] and by high-resolution MS measurement. Acid hydrolysis of 25 with 5% aqueous H2SO4–1,4-dioxane (1:1, v/v) liberated oleanolic acid, d-glucuronic acid, d-xyllose, and d-glucose. The 1H-NMR (pyridine-d7) and 13C-NMR (Table 2) spectra of 25 showed signals assignable to an oleanolic acid part [δ 3.25 (dd, J = 4.0, 11.6 Hz, 3-H), 3.29 (dd, J = 3.7, 13.7 Hz, 18-H), 5.45 (brs, 12-H), β-D-glucopyranosiduronic acid part [δ 5.27 (d, J = 7.3 Hz, GlcA-1-H)], a β-D-glucopyranosyl part [δ 4.94 (d, J = 7.6 Hz, Xyl-1-H)] and a β-D-glucopyranosyl part [δ 5.15 (d, J = 7.6 Hz, Glc-1-H)]. The trigsolide structure bonding to the 3-hydroxyl group of an oleanolic acid moiety in 11 was characterized by a HMBC experiment. Consequently, long-range correlations were observed between the following protons and carbons: GlcA-1-H and 3-C; Xyl-1-H and GlcA-2-C; Glc-1-H and GlcA-4-C; GlcA-3-H and Ac-1-C.

The 1H-NMR (pyridine-d7) and 13C-NMR (Table 2) spectra of 11 showed signals assignable to an acetyl group [δ 2.45 (3H, s, Ac-2)] together with the desacyl-goyasaponin III moiety. Comparison of the 1H- and 13C-NMR data for 11 with those for 25 revealed an acylation shift around the 3-position of the β-D-glucopyranosiduronic acid moiety in the trigsolide part of 11. In the HMBC experiment of 11, a long-range correlation was observed between the 3-proton of the β-D-glucopyranosiduronic acid moiety and the acetyl carbonyl carbon. Consequently, the structure of goyasaponin III has been elucidated as oleanolic acid 3-O-[β-D-glucopyranosyl(1→3)]{β-D-glucopyranosyl(1→2)}3′-O-acetyl-β-D-glucopyranosiduronic acid (11).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were described previously.11

Isolation of Goyaglycosides-a (1), -b (2), -c (3), -d (4), -e (5), -f (6), -g (7), and -h (8). Goyasaponin I (II), and Five Known Glycosides (12—16) from the Fresh Fruit of M. charantia L. (36.9 kg, cultivated in Okinawa prefecture, Japan), which included the immature seeds, was cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (846.0 g, 2.3%), and the extract was further extracted with the n-BuOH. The solvent of the extract under reduced pressure was obtained as the AcOH- and n-BuOH-soluble portions yielding MeOH extract (846.0 g, 2.3%), the extract (828.9 g) was partitioned into the AcOH–H2O (1:1) mixture. The water phase was further extracted with the n-BuOH. The solvent of the extract under reduced pressure was obtained as the AcOH- and n-BuOH-soluble portions yielding 64.5 g (0.17%) and 98.3 (0.27%) of residues, respectively. The n-BuOH-soluble portion (88.3 g) was subjected to normal-phase silica gel column chromatography [BW-200 (Fujisilysia Co., Ltd., 4.5 kg), CHCl3–MeOH–H2O (15:3:1, lower layer), and HPLC [YMPC-Pack ODS-A (YMC Co., Ltd., 1)] to isolate Fraction 1 (2.5 g) was subjected to normal-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fujisilysia Chemical Ltd., 30 g), MeOH–H2O (70:30:20:90:10, v/v/v/v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 1)] to isolate Fraction 1 (2.5 g) was subjected to normal-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fujisilysia Chemical Ltd., 30 g), MeOH–H2O (70:30:20:90:10, v/v/v/v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 1)] to isolate Fraction 1 (2.5 g) was subjected to normal-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fujisilysia Chemical Ltd., 30 g), MeOH–H2O (70:30:20:90:10, v/v/v/v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 1)] to isolate Fraction 1 (2.5 g) was subjected to normal-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fujisilysia Chemical Ltd., 30 g), MeOH–H2O (70:30:20:90:10, v/v/v/v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 1)].
Goyaglycoside-d (1): A white powder, \( [\alpha]_{D}^{27} = -101.4^\circ \) (c = 0.5, MeOH).

High-resolution positive-ion FAB-MS: Caled for C_{38}H_{62}O_{9}Na (M + Na)^{+}: 584.4301, 1.4 ppm. 

\[
\text{Goyaglycoside-b (2): A white powder, } [\alpha]_{D}^{22} = -110.7^\circ \text{ (c = 0.5, MeOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{40}H_{64}O_{10}Na (M + Na)^{+}: 649.4320, 1.6 ppm.

\[\text{Goyaglycoside-c (3): A white powder, } [\alpha]_{D}^{20} = -110.8^\circ \text{ (c = 0.5, MeOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{40}H_{64}O_{10}Na (M + Na)^{+}: 685.4292. 

\[\text{Goyaglycoside-d (4): A white powder, } [\alpha]_{D}^{20} = -141.1^\circ \text{ (c = 0.1, MeOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{40}H_{64}O_{10}Na (M + Na)^{+}: 685.4292. 

\[\text{Goyaglycoside-b (5): A white powder, } [\alpha]_{D}^{20} = -75.6^\circ \text{ (c = 0.5, EtOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{40}H_{64}O_{10}Na (M + Na)^{+}: 781.4739. 

\[\text{Goyaglycoside-c (6): A white powder, } [\alpha]_{D}^{22} = -51.9^\circ \text{ (c = 0.5, EtOH), High-resolution positive-ion FAB-MS: Caled for C_{40}H_{62}O_{10}Na (M + Na)^{+}: 781.4739.}
\]

\[\text{Goyaglycoside-g (7): A white powder, } [\alpha]_{D}^{22} = -79.2^\circ \text{ (c = 0.5, MeOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{40}H_{64}O_{10}Na (M + Na)^{+}: 833.4695. 

\[\text{Goyaglycoside-h (8): A white powder, } [\alpha]_{D}^{22} = +0.7^\circ \text{ (c = 0.5, MeOH).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{38}H_{62}O_{9}Na (M + Na)^{+}: 837.4612. 

\[\text{Goyasaponin I (9): A white powder, } [\alpha]_{D}^{27} = -13.5^\circ \text{ (c = 0.5, pyridine).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{41}H_{66}O_{10}Na (M + Na)^{+}: 1401.6303. 

\[\text{Goyasaponin II (10): A white powder, } [\alpha]_{D}^{22} = -15.0^\circ \text{ (c = 1.0, pyridine).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{41}H_{66}O_{10}Na (M + Na)^{+}: 1533.6725. 

\[\text{Goyasaponin III (11): A white powder, } [\alpha]_{D}^{27} = -0.9^\circ \text{ (c = 0.5, pyridine).}
\]

High-resolution positive-ion FAB-MS: Caled for C_{41}H_{66}O_{10}Na (M + Na)^{+}: 991.4879. 

\[\text{Acid Hydrolysis of Goyaglycosides (1–8), Goyasaponins (9–11), 24, and 25: A solution of 1–11, 24 and 25 (3 mg each) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amber-}

Enzymatic Hydrolysis of Goyaglycosides-a (1) and -c (3) A solution of 1 (9.9 mg) in 0.1 M acetate buffer (pH 4.0, 0.3 ml) was treated with naringinase (Sigma Chemical Co., 30 mg) and was stirred at 40 °C for 10 days. After addition of EtOH to the reaction mixture, the residue was removed in vacuo. The crude product was purified by reversed-phase (2, H<sub>2</sub>O-MeOH) and normal-phase silica gel column chromatography [1g, n-hexane-AcOEt (3:1, v/v)] to give 19(5, 11, 10, 9, 22, 24)-diastereomer (0.1 mg, 1.0% yield) and its 24-diastereomer (0.3 mg, 27.3%), which were identified by comparison with reported values<sup>10</sup> (H-NMR, IR, δ<sub>ν</sub>). 1<sup>h</sup>-H NMR (270 MHz, CDCl<sub>3</sub>): δ 8.59, 8.61 (2H each, s, 21-H), 5.43 (1H, br s, 12-H), 9.92 (1H, s, 23-H). 13C-NMR (68 MHz, CDCl<sub>3</sub>): δ 160.3 (C–MeOH), 160.6 (C–MeOH), 152.6 (C<sub>2</sub>–OH), 152.4 (C<sub>2</sub>–OH), 120.8 (CH<sub>3</sub>–CH=O), 119.3 (CH=O), 64.9 (C<sub>5</sub>–C<sub>22</sub>), 469 (C–MeOH). Positive-ion FAB-MS: m/z 807 [M–H]<sup>+</sup>. 1<sup>h</sup>-H NMR, IR, δ<sub>ν</sub>.

Enzymatic Hydrolysis of Goyaglycoside-b (7) A solution of 7 (0.9 mg) in 0.1 M acetate buffer (pH 4.0, 0.3 ml) was treated with naringinase (Sigma Chemical Co., 30 mg) and was stirred at 40 °C for 10 days. After addition of EtOH to the reaction mixture, the residue was removed in vacuo. The crude product was purified by reversed-phase (2, H<sub>2</sub>O-MeOH) and normal-phase silica gel column chromatography [1g, CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (30:3:1, lower layer, v/v)] to give goyaglycoside-b (2.0 mg, 55.5%), which was identified by comparison with an authentic natural sample (H-NMR, IR, δ<sub>ν</sub>). 1<sup>h</sup>-H NMR (270 MHz, CDCl<sub>3</sub>): δ 8.52, 8.61 (2H each, s, 21-H), 8.03 (1H, d, 23-H), 5.89 (1H, d, 24-H), 1.63 (3H, s, 7-OCH<sub>3</sub>), 1.62 (3H, s, 5-OCH<sub>3</sub>). 1<sup>3</sup>C NMR (68 MHz, CDCl<sub>3</sub>): 163.2 (C<sub>2</sub>–OH), 161.5 (C<sub>2</sub>–OH), 151.8 (C–MeOH), 148.9 (C–MeOH), 120.0 (CH=O), 119.2 (CH=O), 65.1 (C<sub>5</sub>–C<sub>22</sub>), 468 (C–MeOH), 455 (C–MeOH). Positive-ion FAB-MS: m/z 831 [M–H]<sup>+</sup>.

Enzymatic Hydrolysis of Goyaglycoside-c (8) A solution of 8 (9.9 mg) in 0.1 M acetate buffer (pH 4.0, 0.3 ml) was treated with naringinase (Sigma Chemical Co., 30 mg) and was stirred at 40 °C for 24 h. After addition of EtOH to the reaction mixture, the residue was removed in vacuo. The crude product was purified by reversed-phase (2, H<sub>2</sub>O-MeOH) and normal-phase silica gel column chromatography [1g, CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (7:3:1, lower layer, v/v)] to give goyaglycoside-c (2.4 mg, 55.5%), which was identified by comparison with an authentic natural sample (H-NMR, IR, δ<sub>ν</sub>). 1<sup>h</sup>-H NMR (270 MHz, CDCl<sub>3</sub>): δ 8.53, 8.61 (2H each, s, 21-H), 8.04 (1H, d, 23-H), 5.59 (1H, d, 24-H), 1.62 (3H, s, 7-OCH<sub>3</sub>), 1.60 (3H, s, 5-OCH<sub>3</sub>). 1<sup>3</sup>C NMR (68 MHz, CDCl<sub>3</sub>): 162.4 (C<sub>2</sub>–OH), 158.8 (C<sub>2</sub>–OH), 149.6 (C–MeOH), 120.5 (CH=O), 119.5 (CH=O), 65.1 (C<sub>5</sub>–C<sub>22</sub>), 461 (C–MeOH), 443 (C–MeOH). Positive-ion FAB-MS: m/z 873 [M–H]<sup>+</sup>.

References and Notes


7) The 'H- and 13C-NMR spectra of 1—11, 20, 24 and 25 were assigned with the aid of homo- and hetero- correlation spectroscopy ('H-'H, 'H-13C COSY), distortionless enhancement by polarization transfer (DEPT), nuclear Overhauser and exchange spectroscopy (NOESY) and heteronuclear multiple bond correlation (HMBC) experiments.

8) Goyaglycosides-a (1), -b (2), -c (3), -d (4), and -g (7) having one or two methoxyl groups in their structures were believed to be formed during the extraction or isolation procedure. The characterization of their genuine glycosides is an interesting subject for further investigation.
