Medicinal Foodstuffs. IX.[1] The Inhibitors of Glucose Absorption from the Leaves of *Gymnema sylvestre* R. Br. (Asclepiadaceae): Structures of Gymnemosides a and b

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Although the glycosidic fraction from the dried leaves of *Gymnema sylvestre* R. Br., gymnemic acid, was reported to be effective for diabetes, it showed little inhibitory activity on the increase of serum glucose level in oral glucose-loaded rats. From the glycosidic fraction, six triterpene glycosides, gymnemosides a, b, c, d, e, and f, were isolated together with nine known triterpene glycosides. The structures of gymnemosides a and b were determined on the basis of chemical and physicochemical evidence as 21-*O*-tigloyl-22-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid and 16-*O*-acetyl-21-*O*-tigloylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid, respectively. In addition, an acetyl group linked to the 16- or 22-hydroxyl group in gymnemosides a and b was found to migrate easily to the primary 28-hydroxyl group, while the acyl migration from the 28-position was rarely observed.

The inhibitory activity of each triterpene glycoside from gymnemic acid was examined to determine its impact on the increase of serum glucose level in oral glucose-loaded rats. Gymnemoside b and gymnemic acids III, V, and VII were found to exhibit a little inhibitory activity against glucose absorption, but the principal constituents, gymnemic acid I and gymnemasaponin V, lacked this activity.

Key words *Gymnema sylvestre*; gymnemoside; glucose absorption inhibitor; acyl migration; triterpene glycoside; oral glucose-loaded test

The leaves of *Gymnema* (*G.*) *sylvestre* R. Br. (Asclepiadaceae), which is distributed over most of India, are used as a stomachic, a diuretic, and a remedy for cough and eye pain in Indian traditional medicine. This natural medicine is commonly known as "Gur-mar" in India for its distinctive property of temporarily inhibiting the taste of sweetness. The crude saponin fraction of this natural medicine called gymnemic acid was shown not only to suppress the taste of sweetness but also to inhibit glucose absorption in the small intestine of rats in sucrose and glucose tolerance test.[2] Since gymnemic acid is believed to be effective for diabetes and obesity, the leaves of *G. sylvestre* are consumed as a health food in Japan. Many antisweet saponins in gymnemic acid were reported,[3] but the glycoside constituents responsible for the inhibition of glucose absorption have not been identified.[4]

In the course of our studies on the bioactive constituents of medicinal foodstuffs[1,5] and natural medicines,[6] we have characterized many saponins: elatosides, camelliasaponins, escins, senegasaponins betavulgarosides, and scoparianosides, which exhibited potent inhibitory activities on ethanol and glucose absorption in rats, from *Aralia elata* Seem. (root cortex, bark, and young shoots, Araliaceae),[7] *Camellia japonica* L. (seed, Theaceae),[8] *Aesculus hippocastanum* L. (seed, Hippocastanaceae),[9] *Polygala senega* L. var. *latifolia* Torrey *et* Gray (roots, Polygalaceae),[10] *Beta vulgaris* L. (roots and leaves, Chenopodiaceae),[11] and *Kochia scoparia* Schrad. (fruit, Chenopodiaceae).[12] As part of our continuing studies on the saponin constituents with activity to inhibit glucose absorption, we examined the inhibitory activity of the glycosidic fraction and each triterpene glycoside from the leaves of *G. sylvestre* on the increase of serum glucose level in oral glucose-loaded rats. In this paper, we present a full account of the structure elucidation of gymnemosides a (**1**) and b (**2**) and of the effect of the triterpene glycosides from *G. sylvestre* on glucose absorption.[13]

The triterpene glycoside constituents of the leaves of *G. sylvestre* were separated by the procedures shown in Chart 1. Namely, the methanolic extract from the leaves was subjected to reversed-phase silica-gel column chromatography to give the methanol eluate (so-called glycosidic fraction). The methanol eluate was separated by normal-phase silica-gel column chromatography followed by HPLC to provide six new triterpene glycosides called gymnemosides a (**1**, 0.0083%), b (**2**, 0.0046%), c (0.0085%), d (0.0028%), e (0.0019%), and f (0.0018%) together with nine known triterpene glycosides:[3] gymnemic acids I (**3**, 0.012%), II (**4**, 0.0086%), III (**5**, 0.0091%), IV (**6**, 0.0060%), V (**7**, 0.0050%), and VII (**8**, 0.0060%) and gymnemasaponins II (**10**, 0.0025%), IV (**11**, 0.0012%), and V (**12**, 0.016%).

Structures and Acyl Migration of Gymnemosides a (1) and b (2) Gymnemoside a (**1**) was isolated as colorless fine crystals of mp 207.0—208.5 °C. The IR spectrum of **1** showed absorption bands at 3453, 1721, 1649, and 1040 cm^{-1} due to hydroxyl, carboxyl, and ester functions. In the negative-ion and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 805 $(M-H)^-$ and *m/z* 829 $(M+Na)^+$, respectively, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{43}H_{66}O_{14}$. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1, v/v) liberated gymnemagenin 3-*O*-glucuronide (**9**)[3] together with acetic acid and tiglic acid. The

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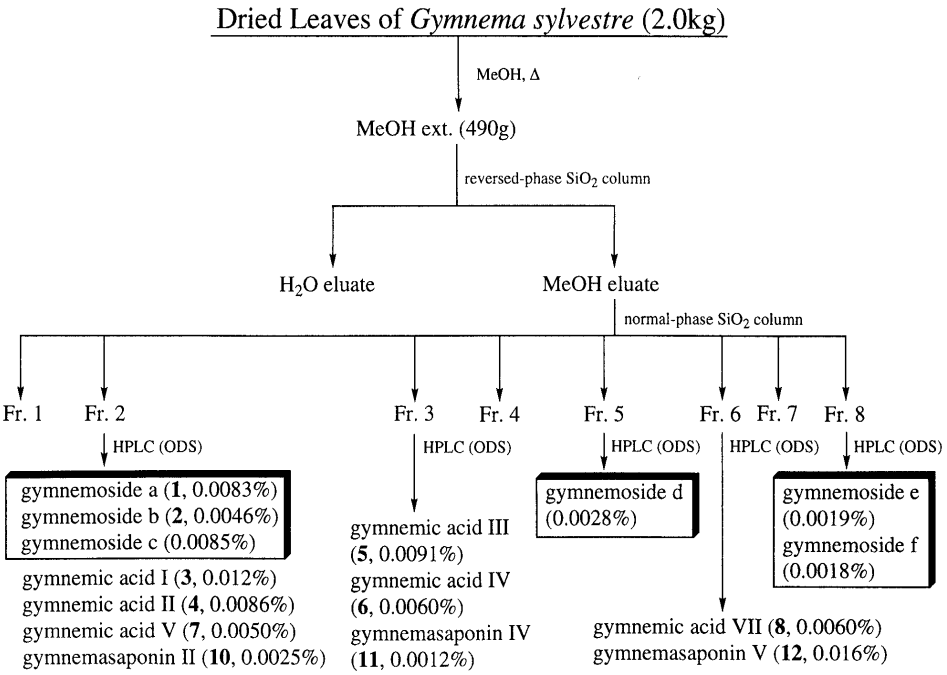
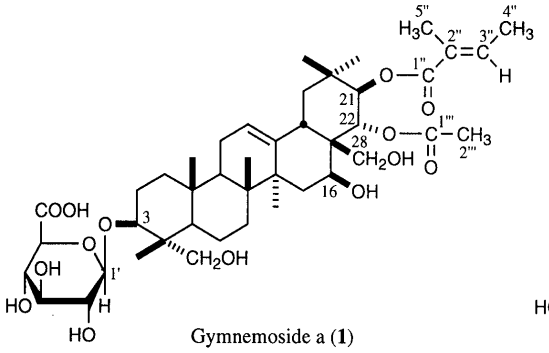
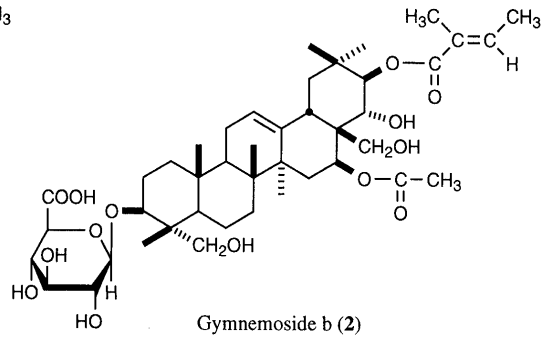


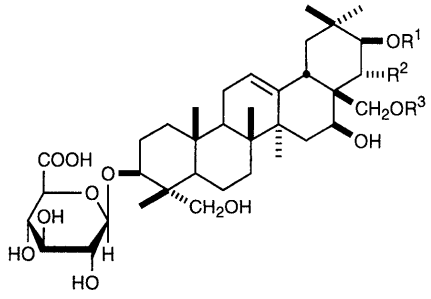
Chart 1



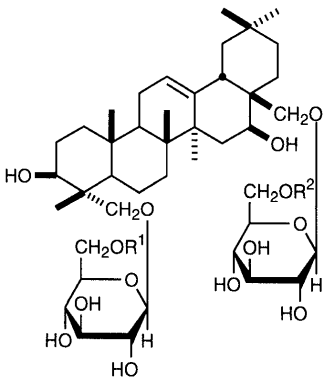
Gymnemoside a (**1**)



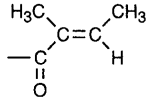
Gymnemoside b (**2**)

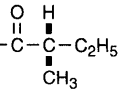


	R^1	R^2	R^3
Gymnemic acid I (**3**) :	Tig	OH	Ac
Gymnemic acid II (**4**) :	MB	OH	Ac
Gymnemic acid III (**5**) :	MB	OH	H
Gymnemic acid IV (**6**) :	Tig	OH	H
Gymnemic acid V (**7**) :	Tig	OTig	H
Gymnemic acid VII (**8**) :	H	H	H
9 :	H	OH	H



	R^1	R^2
Gymnemasaponin II (**10**) :	H	H
Gymnemasaponin IV (**11**) :	Glc	H
Gymnemasaponin V (**12**) :	Glc	Glc

Tig : tigloyl 

MB : (2*S*)-methylbutyroyl 

Glc : β-D-glucopyranosyl

Chart 2

organic acids were converted into the *p*-nitrobenzyl esters, which were identified by HPLC analysis.[3h] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[14] showed signals due to acetyl and tigloyl groups [δ 1.67 (d, J=6.9 Hz, 4″-H_3), 1.95 (br s, 5″-H_3, Ac), 7.12 (dq-like, 3″-H)] and a gymnemagenin 3-*O*-glucuronide moiety [δ 5.28 (d, J=7.3 Hz, 1′-H), 5.77, 6.26 (both d, J=11.2 Hz, 21, 22-H)]. Comparison of the ^{13}C-NMR data for **1** with those for **9** indicated acylation shifts at the 21- and 22-positions. The positions of two acyl groups in **1** were clarified by a heteronuclear multiple bond correlation (HMBC) experiment, in which long-range correlations were observed between the 21-proton and the 1″-carbon of tigloyl group and between the 22-proton and the acetyl carbonyl carbon. Consequently, the chemical structure of gymnemoside a was determined as 21-*O*-tigloyl-22-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid (**1**).

Gymnemoside b (**2**) was also isolated as colorless fine crystals of mp 211.5—213.0 °C and its IR spectrum was very similar to that of **1**. The negative-ion and positive-ion FAB-MS of **2** showed quasimolecular ion peaks at m/z 805 $(M-H)^-$ and m/z 829 $(M+Na)^+$, respectively, and the molecular formula $C_{43}H_{66}O_{14}$, which was the same as that of **1**, was determined by high-resolution MS measurement. Alkaline hydrolysis of **2** gave **9**, acetic acid, and tiglic acid. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra of **2** were almost superimposable on those of **1** and **3**, except for a few signals assignable to the D and E-rings of the gymnemagenin part. Comparison of the ^{13}C-NMR data for **2** with those for **9** showed acylation shift at the 16- and 21-positions. Thus, the carbon signals due to the 16- and 21-carbons were shifted downfield, whereas those due to the 15- and 22-carbons appeared upfield. Furthermore, in the HMBC experiment, long-range correlations were observed between the 16-proton and the acetyl carbonyl carbon and between the 21-proton and the 1″-carbon. On the basis of this evidence, the structure of gymnemoside b was elucidated as 16-*O*-acetyl-21-*O*-tigloylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid (**2**).

Table 1. ^{13}C-NMR Data for Gymnemosides a (**1**) and b (**2**) and Gymnemic Acid I (**3**)

	1	2	3		1	2	3
C-1	38.7	38.7	38.8	C-23	64.4	64.4	64.3
C-2	26.1	26.1	26.0	C-24	13.6	13.6	13.6
C-3	81.9	81.9	82.0	C-25	16.2	16.2	16.2
C-4	43.5	43.5	43.5	C-26	16.9	16.9	17.1
C-5	47.4	47.4	47.4	C-27	27.4	27.4	27.4
C-6	18.0	18.0	18.0	C-28	59.9	59.7	62.3
C-7	32.5	32.5	32.5	C-29	29.2	29.7	29.4
C-8	40.2	40.3	40.2	C-30	19.7	20.1	19.8
C-9	47.1	47.1	47.1	GlcA-1′	106.3	106.3	106.2
C-10	36.8	36.6	36.6	2′	75.5	75.5	75.4
C-11	23.9	23.8	23.9	3′	78.1	78.1	78.1
C-12	124.3	124.3	124.6	4′	73.5	73.4	73.4
C-13	141.5	141.5	141.3	5′	78.0	77.9	77.8
C-14	42.6	42.9	43.5	6′	172.8	172.8	172.8
C-15	36.6	33.6	36.3	Tig-1″	167.8	167.9	168.1
C-16	67.0	69.3	67.5	2″	128.9	129.6	129.5
C-17	47.9	47.4	45.7	3″	137.9	136.3	136.8
C-18	42.6	42.6	42.5	4″	14.3	14.1	14.2
C-19	45.8	46.2	45.7	5″	12.3	12.3	12.4
C-20	36.6	36.7	36.6	Ac-1‴	170.1	170.3	170.9
C-21	76.6	78.8	78.8	2‴	20.9	21.9	20.7
C-22	74.6	70.4	71.6				

(68 MHz, pyridine-d_5)

Since gymnemosides a (**1**) and b (**2**) may be formed from a major saponin constituent gymnemic acid I (**3**), which is their positional isomer of the acetyl group, during the isolation procedure, acyl migration of **1**, **2**, and **3** was examined. Both gymnemosides (**1**, **2**) were heated under reflux for 1 h in 1% aqueous citric acid-methanol (5:95, v/v) solution to give the mixture of **1**, **2**, and **3** in nearly the same ratio (5:4:1 ratio), respectively. Prolongation of reaction time (8 h) increased the proportion of **3** in the mixture of **1**, **2**, and **3** (1:1:8 ratio).[15] In contrast, treatment of **3** under reflux in 1% aqueous citric acid–methanol (5:95, v/v) solution afforded little **1** or **2**. Furthermore, in the HPLC analysis of the methanolic extract of the leaves of *G. sylvestre*, the extract obtained at room temperature gave the mixture of **1**, **2**, and **3** in a 2:1:1 ratio,[15] while that obtained under reflux gave the mixture in a 1:1:4 ratio.[15] **1** and **2** were unchanged in 1% aqueous acetic acid–methanol solution (3:7, v/v) at room temperature, which was used as the elution solvent in the HPLC separation of **1** and **2**. On the basis of those findings, **1** and **2** would not be secondarily formed from **3**, whereas **3** may be generated from **1** and **2** during the extraction under reflux.

Inhibitory Effect of Gymnemosides (1, 2), Gymnemic Acids (3—8), and Gymnemasaponins (10—12) on the Increase of Serum Glucose Level The glycosidic fraction from the leaves of *G. sylvestre* showed no inhibitory effect on the increase of serum glucose level in oral glucose-loaded rats after a single oral administration at various doses (500, 200, 100, 50, and 25 mg), which were previously reported to be effective in oral sucrose- and glucose-loaded test.[2] To closely examine the effectiveness of *G. sylvestre*, each triterpene glycoside from the glycosidic fraction was subjected to the oral glucose-loaded test. With respect to gymnemasaponins II (**10**) and IV (**11**), adequate amounts to examine the activity could not be obtained from the leaves of *G. sylvestre*, so **10** and **11** were prepared from gymnemasaponin V (**12**) by enzymatic hydrolysis using cellulase T-4 and cellulase from *Aspergillus niger*, respectively.

As shown in Table 3, gymnemoside b (**2**) and gymnemic acids III (**5**), V (**7**), and VII (**8**) exhibited slight inhibitory activity on the increase of serum glucose level in oral glucose-loaded rats after a single administration of 100 mg/kg, but other triterpene glycosides including the major components of the glycosidic fraction such as gymnemic acid I (**3**) and gymnemasaponin V (**12**) showed no activity at the same dose. We recently characterized many inhibitors of glucose absorption from medicinal foodstuffs and natural medicines and, by examination of the structure requirement for the activity, active saponins can be classified into the following three types: oleanen-28-oic acid 3-*O*-monodesmoside (elatosides,[7] betavulgarosides,[11] scoparianosides[12]), acylated polyhydroxyoleanene 3-*O*-glucuronide (camelliasaponins,[8]

Table 2. Inhibitory Activity of the Glycosidic Fraction from the Leaves of *G. sylvestre* on the Increase of Serum Glucose Level in Oral Glucose-loaded Rats

	Dose (mg/kg, *p.o.*)	N	Serum Glucose Concentration (mg/dl)		
			0.5 h	1.0 h	2.0 h
Control (normal)	—	4	91.1±3.4**		
Control (glucose-loaded)	—	6	162.1±3.6		
			(71.0±3.6)		
Glycosidic fraction	25	5	156.4±7.4		
			(65.3±7.4)		
	50	5	163.7±3.2		
			(72.6±3.2)		
	100	5	162.2±5.3		
			(71.1±5.3)		
Control (normal)	—	6	68.7±1.8**	98.6±2.7**	89.0±4.1*
Control (glucose-loaded)	—	6	139.9±3.7	145.8±6.6	114.0±6.7
			(71.2±3.7)	(47.2±6.6)	(25.0±6.7)
Glycosidic fraction	200	6	133.9±6.4	136.5±3.8	101.3±6.1
			(65.2±6.4)	(37.9±3.8)	(12.3±6.1)
Control (normal)	—	5	79.0±2.4**	94.0±2.1**	87.8±2.5
Control (glucose-loaded)	—	6	136.3±4.4	121.3±4.8	94.5±2.4
			(57.3±4.4)	(27.3±4.8)	(6.7±2.4)
Glycosidic fraction	500	5	130.0±8.0	129.6±4.9	98.0±3.6
			(51.0±8.0)	(35.6±4.9)	(10.2±3.6)

*$p<0.05$, **$p<0.01$. Values in parenthesis show the difference in serum glucose concentration between the normal control and each sample treatment.

Table 3. Inhibitory Effects of Gymnemosides (**1**, **2**), Gymnemic Acid (**3**—**8**), and Gymnemasaponins (**10**—**12**) from the Leaves of *G. sylvestre* on the Increase of Serum Glucose Level in Oral Glucose-loaded Rats

	Dose (mg/kg, *p.o.*)	N	Serum Glucose Concentration (mg/dl)		
			0.5 h	1.0 h	2.0 h
Control (normal)	—	4	73.5±2.5**	98.9±8.9**	89.6±5.9*
Control (glucose-loaded)	—	5	150.9±7.6	143.3±6.1	113.1±2.1
			(77.4±7.6)	(44.4±6.1)	(23.5±2.1)
Gymnemoside a (**1**)	100	5	146.3±2.6	143.9±4.5	112.8±5.6
			(72.8±2.6)	(45.0±4.5)	(23.2±5.6)
Gymnemoside b (**2**)	100	4	134.7±7.6	139.3±6.4	115.4±4.5
			(61.2±7.6)	(40.4±6.4)	(25.8±4.5)
Control (normal)	—	5	81.3±7.4**	97.5±7.8**	92.1±4.7**
Control (glucose-loaded)	—	5	146.8±4.7	142.2±5.0	111.7±2.6
			(65.5±4.7)	(44.7±5.0)	(19.6±2.6)
Gymnemic acid I (**3**)	100	5	146.8±6.4	138.3±6.2	102.3±2.3*
			(65.5±6.4)	(40.8±6.2)	(10.2±2.3*)
Gymnemic acid II (**4**)	100	5	148.3±3.5	145.5±4.2	109.9±4.2
			(67.0±3.5)	(48.0±4.2)	(17.8±4.2)
Gymnemic acid V (**7**)	100	5	123.2±6.1*	127.4±5.0	110.1±2.1
			(41.9±6.1*)	(29.9±5.0)	(18.0±2.1)
Control (normal)	—	5	79.9±4.4**	97.6±2.4**	94.0±2.1**
Control (glucose-loaded)	—	10	142.8±4.3	130.9±3.6	113.1±4.0
			(62.9±4.3)	(33.3±3.6)	(19.1±4.0)
Gymnemic acid III (**5**)	100	4	126.2±6.9	150.8±5.3*	120.1±7.1
			(46.3±6.9)	(53.2±5.3*)	(26.1±7.1)
Gymnemic acid IV (**6**)	100	5	150.8±5.1	149.7±3.6**	111.1±3.7
			(70.9±5.1)	(52.1±3.6**)	(17.1±3.7)
Gymnemasaponin II (**10**)	100	5	132.4±4.6	136.8±3.1	117.3±7.5
			(52.5±4.6)	(39.2±3.1)	(23.3±7.5)
Gymnemasaponin IV (**11**)	100	3	135.7±1.8	137.4±0.5	113.4±3.7
			(55.8±1.8)	(39.8±0.5)	(19.4±3.7)
Gymnemasaponin V (**12**)	100	5	139.9±3.3	138.1±6.9	115.2±4.0
			(60.0±3.3)	(40.5±6.9)	(21.2±4.0)
Control (normal)	—	5	70.6±3.7**	90.6±6.1**	73.9±4.7**
Control (glucose-loaded)	—	5	137.5±2.3	125.0±4.8	97.9±2.3
			(66.9±2.3)	(34.4±4.8)	(24.0±2.3)
Gymnemic acid VII (**8**)	100	7	122.1±5.2*	113.4±6.3	97.1±3.5
			(51.5±5.2*)	(22.8±6.8)	(23.2±3.5)

*$p<0.05$, **$p<0.01$. Values in parenthesis show the difference in serum glucose concentration between the normal control and each sample treatment.

escins[9]), and oleanene 3,28-*O*-acylated bisdesmoside (senegins, senegasaponins[10]). Although the active glycosides (**2**, **5**, **7**, **8**) from *G. sylvestre* are classified into the acylated polyhydroxyoleanene 3-*O*-glucuronide group, it is noted that the inhibitory activities of elatosides, betavulgarosides, escins, camelliasaponins, senegasaponins are much more potent than those of **2**, **5**, **7**, and **8** in oral glucose-loaded test.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as we described previously.[5,6]

Isolation of Gymnemosides a (1), b (2), c, d, e, and f and Known Compounds (3—8, 10—12) from the Leaves of *Gymnema sylvestre* The dried leaves of *Gymnema sylvestre* (2 kg, cultivated in India and purchased from Itou Kanpo Seiyaku, Ltd., Osaka, Lot. No. 25889) were cut finely and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (492 g, 24.6%). This extract was subjected to reversed-phase silica-gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 2 kg), $H_2O \rightarrow$MeOH] to give the H_2O eluate and the MeOH eluate (240 g, 12.0%). Normal-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 3 kg), $CHCl_3$: MeOH : H_2O (7 : 3 : 0.5 $\rightarrow$ 6 : 4 : 1)] of the MeOH eluate (240 g) give eight fractions [fr. 1 (38.1 g), fr. 2 (14.5 g), fr. 3 (52.3 g), fr. 4 (69.7 g), fr. 5 (17.8 g), fr. 6 (10.0 g), fr. 7 (19.4 g), fr. 8 (12.5 g)]. Fraction 2 (1.0 g) was separated by reversed-phase silica-gel column chromatography [290 g, MeOH : H_2O (60 : 40 $\rightarrow$ 75 : 25, v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH : 1% aqueous AcOH (75 : 25, v/v)] to give gymnemosides a (**1**, 166 mg, 0.0083%), b (**2**, 92 mg, 0.0046%), and c (171 mg, 0.0085%), gymnemic acids I (**3**, 240 mg, 0.012%), II (**4**, 172 mg, 0.0086%), and V (**7**, 100 mg, 0.0050%), and gymnemasaponin II (**10**, 50 mg, 0.0025%). Fraction 3 (500 mg) was purified by HPLC [MeOH : 1% aqueous AcOH (70 : 30, v/v)] to give gymnemic acids III (**5**, 181 mg, 0.0091%) and IV (**6**, 120 mg, 0.0060%) and gymnemasaponin IV (**11**, 24 mg, 0.0012%). Fraction 5 (500 mg) was purified by HPLC [MeOH : 1% aqueous AcOH (70 : 30, v/v)] to give gymnemoside d (56 mg, 0.0028%). Fraction 6 (500 mg) was purified by HPLC [MeOH : 1% aqueous AcOH (60 : 40, v/v)] to give gymnemic acid VII (**8**, 119 mg, 0.0060%) and gymnemasaponin V (**12**, 326 mg, 0.0163%). Fraction 8 (2 g) was separated by reversed-phase silica-gel column chromatography [40 g, MeOH : H_2O (60 : 40, v/v) $\rightarrow$ MeOH] and HPLC [MeOH : 1% aqueous AcOH (65 : 35, v/v)] to give gymnemosides e (37 mg, 0.0019%) and f (36 mg, 0.0018%). The known compounds (**3**—**8**, **10**—**12**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.

Gymnemoside a (**1**): Colorless fine crystals from aqueous MeOH, mp 207.0—208.5 °C, $[\alpha]_D^{29}$ +4.7° (c = 0.1, MeOH). High-resolution negative-ion FAB-MS m/z: Calcd for $C_{43}H_{65}O_{14}$ $(M-H)^-$: 805.4375. Found: 805.4385. High-resolution positive-ion FAB-MS m/z: Calcd for $C_{43}H_{66}O_{14}Na$ $(M+Na)^+$: 829.4350. Found: 829.4430. IR (KBr) cm^{-1}: 3453, 1721, 1649, 1040. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 0.90, 0.94, 0.98, 1.03, 1.27, 1.30 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3), 1.67 (3H, d, J = 6.9 Hz, Tig-4″-H_3), 1.95 (6H, br s, Tig-5″-H_3, Ac-2‴-H_3), 3.08 (1H, dd-like, 18-H), 3.73, 4.37 (1H each, both d, J = 10.9 Hz, 23-H_2), 4.01, 4.25 (2H, ABq, J = 10.9 Hz, 28-H_2), 4.36 (1H, m, 3-H), 5.03 (1H, dd-like, 16-H), 5.28 (1H, d, J = 7.3 Hz, GlcA-1′-H), 5.36 (1H, br s, 12-H), 5.77 (1H, d, J = 11.2 Hz, 21-H), 6.26 (1H, d, J = 11.2 Hz, 22-H), 7.12 (1H, dq-like, Tig-3″-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 805 $(M-H)^-$. Positive-ion FAB-MS m/z: 829 $(M+Na)^+$.

Gymnemoside b (**2**): Colorless fine crystals from aqueous MeOH, mp 211.5—213.0 °C, $[\alpha]_D^{29}$ +6.6° (c = 0.1, MeOH). High-resolution negative-ion FAB-MS m/z: Calcd for $C_{43}H_{65}O_{14}$ $(M-H)^-$: 805.4375. Found: 805.4404. High-resolution positive-ion FAB-MS m/z: Calcd for $C_{43}H_{66}NaO_{14}$ $(M+Na)^+$: 829.4350. Found: 829.4428. IR (KBr) cm^{-1}: 3445, 1718, 1649, 1044. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 0.90, 0.93, 1.21, 1.39 (3H each, all s, 25, 26, 30, 27-H_3), 0.98 (6H, s, 24, 29-H_3), 1.56 (3H, d, J = 7.3 Hz, Tig-4″-H_3), 1.78 (3H, s, Tig-5″-H_3), 2.07 (3H, s, Ac-2‴-H_3), 3.15 (1H, dd-like, 18-H), 3.73, 4.37 (1H each, both d, J = 10.9 Hz, 23-H_2), 4.07, 4.65 (1H each, both d, J = 10.2 Hz, 28-H_2), 4.34 (1H, m, 3-H), 4.88 (1H, d, J = 10.9 Hz, 22-H), 5.27 (1H, d, J = 7.6 Hz, GlcA-1′-H), 5.35 (1H, br s, 12-H), 5.75 (1H, d, J = 10.9 Hz, 21-H), 6.40 (1H, dd-like, 16-H), 6.92 (1H, dq-like, Tig-3″-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 805 $(M-H)^-$. Positive-ion FAB-MS m/z: 829 $(M+Na)^+$.

Alkaline Hydrolysis of Gymnemosides a (1) and b (2) A solution of **1** or **2** (20 mg each) in 10% aqueous KOH : 50% aqueous dioxane (1 : 1, v/v, 2 ml) was stirred at 37 °C for 1 h. The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was filtered off. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by normal-phase silica-gel column chromatography [2 g, $CHCl_3$: MeOH : H_2O (6 : 4 : 1)] to give gymnemagenin 3-*O*-glucuronide (**9**, 13.4 mg from **1**, 14.6 mg from **2**) and an organic acid fraction. A solution of the organic acid fraction (1 mg each) in dichloroethane (2 ml) was treated with *p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (10 mg) and the reaction mixture was stirred at 80 °C for 1 h. After cooling, the reaction mixture was subjected to HPLC analysis [column, TSK-gel ODS-Prep (250 × 4.6 mm i.d.); solvent, MeOH : H_2O (70 : 30, v/v); flow rate, 1.0 ml/min] to identify *p*-nitrobenzylacetate [t_R 8.0 min] and *p*-nitobenzyltiglate [t_R 17.0 min].

Acyl Migration of Gymnemosides a (1) and b (2) and Gymnemic Acid I (3) i) A solution of **1**, **2**, or **3** (0.5 mg each) in 1% aqueous citric acid : MeOH (5 : 95, v/v, 0.1 ml) was heated under reflux for 1 or 8 h. The reaction solution was subjected to HPLC analysis [column, YMC-Pack R&D R-ODS-5-A (YMC Co., Ltd., 250 × 4.6 mm i.d.); solvent, MeOH : 1% aqueous AcOH (70 : 30, v/v); flow rate, 1.0 ml/min].

ii) A solution of **1**, **2**, and **3** (0.6 mg each) in 1% aqueous AcOH : MeOH (30 : 70, v/v, 0.12 ml) was stirred at room temperature (25 °C) for 10 h. The reaction solution was subjected to HPLC analysis (the same conditions as described above).

iii) Leaves of *G. sylvestre* (powder, 10 g) were extracted with MeOH for 30 min at room temperature (25 °C) or under reflux. The extract was subjected to HPLC analysis [column, YMC-Pack ODS-AQ (YMC Co., Ltd., 250 × 4.6 mm i.d.); solvent, MeOH : pH 2.6 phosphate buffer (70 : 30, v/v); flow rate, 1.0 ml/min].

Partial Hydrolysis of Gymnemasaponin V (12) with Cellulase T-4 A solution of **12** (180.0 mg) in 0.1 M acetate buffer (pH 4.6, 20 ml) was treated with cellulase T-4 (Amano Seiyaku Co., Ltd., 200 mg) and the reaction mixture was stirred at 37 °C for 3 h. After treatment of the reaction solution with EtOH, the entire mixture was concentrated under reduced pressure to give a residue, which was purified by normal-phase silica-gel column chromatography [30 g, $CHCl_3$: MeOH : H_2O (7 : 3 : 1, lower layer $\rightarrow$ 65 : 35 : 10, lower layer)] to give gymnemasaponin II (**10**, 840 mg). The gymnemasaponin II was identical with an authentic sample by TLC, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR spectra comparisons.

Partial Hydrolysis of Gymnemasaponin V (12) with Cellulase A solution of **12** (216.4 mg) in 0.1 M acetate buffer (pH 5.0, 43 ml) was treated with cellulase from *Aspergillus niger* (Sigma, 217 mg) and the entire mixture was stirred at 37 °C for 1 h. The reaction mixture was treated with EtOH and then the whole was concentrated under reduced pressure to give a residue. The residue was purified with HPLC [MeOH : 1% aqueous AcOH (3 : 1, v/v)] to give gymnemasaponin IV (**11**, 48.2 mg), which was identical with an authentic sample by TLC, HPLC, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR spectra comparisons.

Bioassay of the Inhibitory Activity on the Increase of Serum Glucose Level in Oral Glucose-loaded Rats Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 125—155 g were starved for 20—24 h but allowed water *ad libitum*. The test samples were dissolved in water (5 ml/kg) and orally administered to the rats at each dose. Thirty min thereafter, a water solution (5 ml/kg) of sucrose (0.5 g/kg) was orally administered. Blood (0.4 ml) was collected from the carotid at 0.5, 1.0, and 2.0 h after D-glucose administration and the plasma glucose concentration was assayed by the enzymatic glucose oxidase method. Statistical significance of differences was estimated by analysis of variance (ANOVA) followed by Dunnett's test.[16] Results were expressed as the mean ± S. E. (Tables 2 and 3).

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References and Notes

1) Part VIII : Yoshikawa M., Murakami T., Komatsu H., Yamahara

J., Matsuda H., *Heterocycles*, **47** (1) (1998), in press [No. COM-97-S (N) 52].
2) *a*) Yoshioka S., Takeuchi T., Imoto T., Kasagi T., Hiji Y., *Igakunoayumi*, **135**, 241—242 (1985); *b*) Yoshioka S., *J. Yonago Ned. Ass.*, **37**, 142—154 (1986); *c*) Kurata Y., *ibid.*, **38**, 61—70 (1987); *d*) Ikeuchi H., *ibid.*, **41**, 414—431 (1990); *e*) Hirata S., *ibid.*, **43**, 350—364 (1992); *f*) Hirata S., Abe T., Imoto T., *ibid.*, **43**, 392—396 (1992); *g*) Hirata S., Terasawa H., Katou T., Imoto T., *ibid.*, **43**, 397—404 (1992).
3) *a*) Kurihara Y., *Life Sciences*, **8**, 537—543 (1969); *b*) Tsuda Y., Kiuchi F., Liu H. M., *Tetrahedron Lett.*, **30**, 361—362 (1989); *c*) Yoshikawa K., Amimoto K., Arihara S., Matsuura K., *ibid.*, **30**, 1103—1106 (1989); *d*) *Idem*, *Chem. Pharm. Bull.*, **37**, 852—854 (1989); *e*) Kiuchi F., Liu H. M., Tsuda Y., *ibid.*, **38**, 2326—2328 (1990); *f*) Yoshikawa K., Arihara S., Matsuura K., *Tetrahedron Lett.*, **32**, 789—792 (1991); *g*) Liu H. M., Kiuchi F., Tsuda Y., *Chem. Pharm. Bull.*, **40**, 1366—1375 (1992); *h*) Yoshikawa K., Nagasawa M., Yamamoto R., Arihara S., Matsuura K., *ibid.*, **40**, 1779—1782 (1992).
4) *a*) Recently, a known cyclitol conduritol A was isolated from the leaves of *G. sylvestre* as an inhibitor of intestinal glucose absorption[4b,c]; *b*) Miyatake K., Takenaka S., Fujimoto T., Kensho G., Upadhaya S. P., Kirihata M., Ichimoto I., Nakano Y., *Biosci. Biotech. Biochem.*, **57**, 2184—2185 (1993); *c*) Miyatake K., Kensho G., Fujimoto T., Noguchi E., Shinohara M., Takenaka S., Taira T., Upadhaya S. P., Ichimoto I., Nakano Y., *ibid.*, **58**, 753—757 (1994).
5) *a*) Yoshikawa M., Yoshizumi S., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 492—499 (1996); *b*) Yoshikawa M., Murakami T., Komatsu H., Murakami N., Yamahara J., Matsuda H., *ibid.*, **45**, 81—87 (1997); *c*) Yoshikawa M., Shimada H., Saka M., Yoshizumi S., Yamahara J., Matsuda H., *ibid.*, **45**, 464—469 (1997); *d*) Yoshikawa M., Shimada H., Komatsu H., Sakurama T., Nishida N., Yamahara J., Shimoda H., Matsuda H., Tani T., *ibid.*, **45**, 877—882 (1997).
6) *a*) Yoshikawa M., Shimada H., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 1656—1662 (1996); *b*) Yoshikawa M., Murakami T., Ueda T., Matsuda H., Yamahara J., Murakami N., *ibid.*, **44**, 1736—1743 (1996); *c*) Yoshikawa M., Shimada H., Shimoda H., Murakami N., Yamahara J., Matsuda H., *ibid.*, **44**, 2086—2091 (1996); *d*) Yoshikawa M., Murakami T., Ueda T., Yoshizumi S., Ninomiya K., Murakami N., Matsuda H., Saito M., Fuji W., Tanaka T., Yamahara J., *Yakugaku Zasshi*, **117**, 108—118 (1997); *e*) Yoshikawa M., Murakami T., Ueno T., Yashiro K., Hirokawa N., Murakami N., Yamahara J., Matsuda H., Saijoh R., Tanaka O., *Chem. Pharm. Bull.*, **45**, 1039—1045 (1997); *f*) Yoshikawa M., Murakami T., Ueno T., Hirokawa N., Yashiro K., Murakami N., Yamahara J., Matsuda H., Saijoh R., Tanaka O., *ibid.*, **45**, 1056—1062 (1997).
7) *a*) Yoshikawa M., Yoshizumi S., Ueno T., Matsuda H., Murakami T., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 1878—1882 (1995); *b*) Yoshikawa M., Murakami T., Harada E., Murakami N., Yamahara J., Matsuda H., *ibid.*, **44**, 1915—1922 (1996); *c*) *Idem*, *ibid.*, **44**, 1923—1927 (1996).
8) *a*) Yoshikawa M., Harada E., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 742—744 (1994); b) Yoshikawa M., Murakami T., Yoshizumi S., Murakami N., Yamahara J., Matsuda H., *ibid.*, **44**, 1899—1907 (1996).
9) *a*) Yoshikawa M., Murakami T., Matsuda H., Yamahara J., Murakami N., Kitagawa I., *Chem. Pharm. Bull.*, **44**, 1454—1464 (1996); *b*) Matsuda H., Li Y., Murakami T., Ninomiya K., Araki N., Yoshikawa M., Yamahara J., *Bioorg. Med. Chem. Lett.*, **7** (1997) in press (No. BMCL-97039).
10) *a*) Yoshikawa M., Murakami T., Ueno T., Kadoya M., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 350—352 (1995); *b*) *Idem*, *ibid.*, **43**, 2115—2122 (1995); *c*) Yoshikawa M., Murakami T., Matsuda H., Ueno T., Kadoya M., Yamahara J., Murakami N., *ibid.*, **44**, 1305—1313 (1996).
11) *a*) Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Yamahara J., Muraoka O., Murakami N., *Heterocycles*, **41**, 1621—1626 (1995); *b*) Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Muraoka O., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 1212—1217 (1996); *c*) Yoshikawa M., Murakami T., Inaduki M., Hirano K., Yamahara J., Matsuda H., *ibid.*, **45**, 561—563 (1997).
12) *a*) Yoshikawa M., Yamahara J., "Saponins Used in Traditional and Modern Medicine," ed. by Waller G. R., Yamasaki K., Plenum Press, New York, pp. 207—218, 1996; *b*) Yoshikawa M., Dai Y., Shimada H., Morikawa T., Matsumura N., Yoshizumi S., Matsuda Hisa., Matsuda Hide., Kubo M., *Chem. Pharm. Bull.*, **45**, 1052—1055 (1997); *c*) Yoshikawa M., Shimoda H., Morikawa T., Yoshizumi S., Matsumura N., Murakami T., Matsuda H., Hori K., Yamahara J., *ibid.*, **45**, 1300—1305 (1997); *d*) Matsuda H., Murakami T., Shimada H., Matsumura N., Yoshikawa M., Yamahara J., *Biol. Pharm. Bull.*, **20**, 717—719 (1997).
13) *a*) Part of this work was reported in our preliminary communication[13b]; *b*) Murakami N., Murakami T., Kadoya M., Matsuda H., Yamahara J., Yoshikawa M., *Chem. Pharm. Bull.*, **44**, 469—471 (1996).
14) The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were assigned with the aid of homo and hetero correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), homo and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA), and HMBC experiments.
15) The proportions of **1**, **2**, and **3** were determined from the peak areas in HPLC analysis.
16) *a*) Dunnett C. W., *J. Am. Statist. Assoc.*, **75**, 789—795 (1980); *b*) *Idem*, *ibid.*, **75**, 796—800 (1980).