Isolation and Structure Elucidation of Gymnemic Acids, Antisweet Principles of *Gymnema sylvestre*

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The structure of gymnemagenin (3β,16β,21β,22α,23,28-hexahydroxy-olean-12-ene), the sapogenin of the antisweet principles of *Gymnema sylvestre*, was established by X-ray analysis of the 3β,23;21β,22α-di-*O*-isopropylidene derivative. On the basis of this result, the structure of deacylgymnemic acid was elucidated as the 3-*O*-β-glucuronide from the carbon-13 nuclear magnetic resonance spectra.

Five antisweet principles, gymnemic acid-III, -IV, -V, -VIII, and -IX, were isolated in pure states from the hot water extract of leaves of *Gymnema sylvestre*. Of these, three (GA-III, -IV, and -V) were known, while two (GA-VIII and -IX) were new compounds. The structures of GA-VIII and -IX were elucidated as 3′-*O*-β-D-*arabino*-2-hexulopyranosyl gymnemic acid-III and -IV, respectively.

Keywords *Gymnema sylvestre*; Asclepiadaceae; antisweet activity; gymnemic acid; saponin; gymnemic acid-VIII; gymnemic acid-IX; oxo-glycoside; D-*arabino*-hexopyranosid-2-ulose

Gymnema sylvestre R. BR. is a stout, large, woody climbing plant belonging to the family Asclepiadaceae. In India leaves of this plant have been used as a stomachic, a diuretic, and to treat cough, throat trouble, and eye pain.[1] In addition, this plant is famous for its strange ability to antagonize the sweet taste of sugar: after chewing one or two leaves, one is unable to detect the sweet taste. The Hindi name "Gur-mar" of this plant, meaning sugar-destroying, is attributed to this unique property. The antisweet activity of this plant first appeared in western literature[2] in 1847, and has been experimentally verified by many investigators: gymnema leaves prevent the sweet taste in all regions of the mouth and this taste suppression is believed to involve direct interaction with the taste buds.[1]

As regards the pharmacological actions, Gharpurey[3] (1926) suggested that water extract of the leaves was effective to treat diabetes mellitus. Since then, much work has been done on the effect of *Gymnema sylvestre* on blood sugar in animals and humans. Recently Hiji's group[4] reported that the water extract of gymnema leaves inhibited absorption of glucose in the small intestine and suppressed the increase of blood glucose value after administration of sucrose in rat. An antieurodonic effect[4e] of gymnema extract and an antiviral effect[5] were also suggested.

As early as 1887, Hooper[6] noted that the antisweet principle could be precipitated from the water extract of the leaves by acidification with mineral acid, and he named it gymnemic acid. Boiling with dilute hydrochloric acid destroyed the antisweet property of gymnemic acid. In 1959, Warren and Pfaffmann[7] obtained gymnemic acid in a microcrystalline form and considered that it was a glycoside, because it yielded glucose, arabinose, and a small amount of glucuronolactone upon acid hydrolysis. Later, Yackzan[8] (1966) also suggested that it could be a saponin because of its glycosidic nature, its foaming and hemolytic properties of the aqueous solution.

In 1967, Stöcklin[9] separated gymnemic acid (abbreviated as GA hereafter) into four components, A_1—A_4, and reported that they were D-glucuronides of a hexahydroxy-olean-12-ene named gymnemagenin, being differently acylated with various combinations of formic, acetic, *n*-butyric, isovaleric, and tiglic acid. Sinsheimer *et al.*[10] isolated five gymnemic acids, A, B, C, D, and V, of which A and B were identical with Stöcklin's GA-A_1 and A_2, respectively, by direct thin layer chromatography (TLC) comparisons in five different solvent systems. In 1969, Kurihara[11] isolated GA-A_1 by chromatography on a diethylaminoethyl (DEAE)-Sephadex A-25 column using 95% ethanol as an eluant. This GA-A_1 could be converted into A_2 by potassium bicarbonate hydrolysis and into A_3 by potassium hydroxide hydrolysis. She also reported that A_1, A_2, and A_3 were the same as Stöcklin's GA-A_1, A_2, and A_3, respectively. Dateo and Long[12] reported that GA-A_1 was still a mixture, which could be separated into GA-A_{11} and A_{12}. Chakravarti and Debnath[13] also isolated gymnemic acid, which gave gymnemagenin, glucuronic acid, and galactose on alkaline hydrolysis followed by acid treatment. The above historical survey is summarized in

TABLE I. Studies on Gymnemic Acid before 1985

	Compound	Genin	Acyl moieties	Sugars	Reference
Hooper (1887)	Gymnemic acid				6
Warren, Pfaffmann (1959)	Gymnemic acid			Glucose, arabinose Glucuronolactone	7
Yackzan (1966)	Gymnemic acid				8
Stöcklin (1967)	Gymnemic acid A_1, A_2, A_3, A_4	Gymnemagenin	Formyl, acetyl, butyryl tigloyl, isovaleryl	Glucuronic acid	9
Sinsheimer *et al.* (1968)	A, B, C,[a] D,[a] V	Gymnemagenin Gymnestrogenin	Formyl, acetyl, feruloyl Isovaleryl, tigloyl	Glucuronic acid	10
Kurihara (1969)	A_1	Gymnemagenin	Acetyl, tigloyl, isovaleryl	Glucuronic acid	11
Dateo, Long (1973)	A_{11}, A_{12}	Gymnemagenin	Tigloyl	Glucuronic acid	12
Chakravarti, Debnath (1980)	Gymnemic acid	Gymnemagenin		Glucuronic acid, galactose	13

a) Saponins possessing gymnestrogenin (16-deoxygymnemagenin) as a genin.

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Table I.

The structure elucidation of gymnemagenin was carried out by Stöcklin[9,14)] and Rao and Sinsheimer.[15)] Hydrolysis of gymnemic acid mixture by β-glucuronidase followed by alkaline hydrolysis gave gymnemagenin, mp 328—335 °C,[9)] which was elucidated to be 3β,16β,21β,22α,23,28-hexahydroxyolean-12-ene (**1**), mainly from the data obtained on the genin and its derivatives by physical methods. Treatment of gymnemagenin with acetone–$CuSO_4$ gave two diacetonides, A (mp 305—307 °C) and B (mp 280—281 °C).[14b)] Rao and Sinsheimer[15)] supported Stöcklin's structure of gymnemagenin and they obtained a new diacetonide C (mp 301—303 °C) by treatment of gymnemagenin with acetone–H_2SO_4. Diacetonide C slowly absorbed 1 mol of periodate, suggesting the presence of a 1,2-*trans*-glycol system. The structures of these diacetonides were supposed to be as shown in Chart 1. Dateo and Long[12)] proposed that gymnemic acid is an acylated gymnemagenin 3-*O*-β-D-glucuronide, but no experimental evidence was given for this structure.

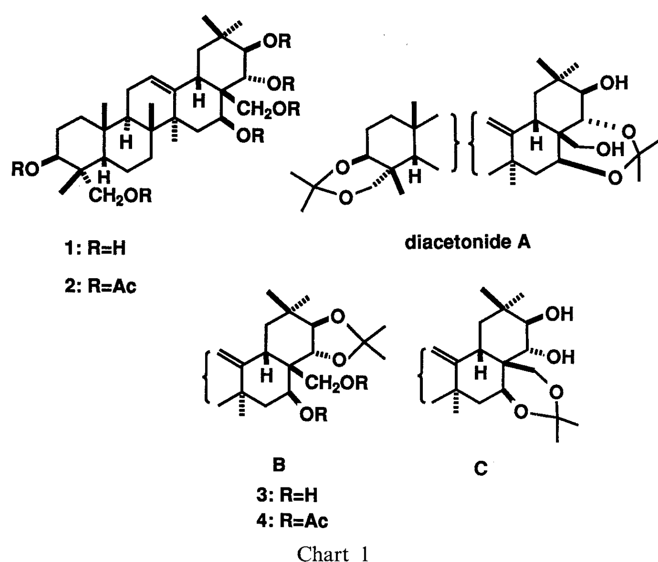

Chart 1

Recently, Yoshikawa *et al.*[16)] isolated six gymnemic acids (GA-I—VI) in pure states by chromatography of water extract of the leaves on a silica gel column and an octadecyl silica (ODS) column. All six are 3-*O*-β-D-glucuronides of gymnemagenin differently acylated by acetyl and/or tigloyl and 2-methylbutyryl groups. Their structures were elucidated on the basis of two-dimensional nuclear magnetic resonance (2D-NMR) and mass spectral analyses and the absolute configuration of the 2-methylbutyryl group was determined as (*S*) by isolation of (*S*)-2-methylbutyric acid from the alkaline hydrolyzate. In the same year, Kurihara *et al.*[17)] reported the isolation and structure elucidation of chromatographically pure GA-I and GA-II, which appeared to be identical with Yoshikawa's GA-IV and GA-III, respectively.

Since the structure elucidations of these authors were based on Stöcklin's gymnemagenin structure, and since the structure has not yet been established completely, as Stöcklin himself stated,[1)] a definitive structure proof of gymnemagenin is urgently required.

In this paper we firstly present a proof of the structure of gymnemagenin by X-ray analysis,[18)] then describe our independent work on the isolation and structure determination of five gymnemic acids[19)] including two new congeners GA-VIII and -IX, both of which contain a hexulopyranoside moiety.[20)] The gymnemic acids so far isolated in a pure state and structurally determined are listed in Table II.

Results and Discussion

X-Ray Determination of the Structure of Gymnemagenin A crude saponin mixture of *G. sylvestre* was obtained from a hot water extract of the leaves by Kurihara's procedure.[11)] Treatment of the crude saponin with 3% KOH–MeOH, gave deacylgymnemic acid (DAGA) (**5**), mp 230—235 °C, as a microcrystalline compound. It formed a methyl ester **6** on treatment with diazomethane. The fast atom bom-

TABLE II. Gymnemic Acids (GA) so far Characterized as Pure Compounds

5: $R^1=R^2=R^3=R^4=R^5=H$
6: $R^1=R^2=R^3=R^4=H$, $R^5=Me$

This work		Arihara's group[a)]		Kurihara's group[b)]		Structure					
GA	mp (°C)	GA	mp (°C)	GA	mp (°C)		R^1	R^2	R^3	R^4	R^5
		I	(211—212)[c)]			**7**	Tig	H	Ac	H	H
		II	(212—213)			**8**	MB	H	Ac	H	H
III	219—221	III	(218—219)	II	(209.5—212.0)	**9**	MB	H	H	H	H
IV	229—231	IV	(220—221)	I	(213.0—215.0)	**10**	Tig	H	H	H	H
V	214—216	V	(202—203)			**11**	Tig	Tig	H	H	H
		VI	(225—226)			**12**	Tig	H	H	β-Glc	H
VIII	218—220					**13**	MB	H	H	OG	H
IX	222—224					**14**	Tig	H	H	OG	H

Tig = Tigloyl. MB = (*S*)-2-Methylbutyryl. OG = β-*arabino*-2-Hexulopyranosyl. *a*) Ref. 16. *b*) Ref. 17. *c*) Parenthetical values show reported melting points.

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TABLE III. ^{13}C-NMR Data for Gymnemic Acids and Derivatives[a)]

No.	Genin **1**	DAGA **5**	Me DAGA **6**	GA-III **9**	GA-IV **10**	GA-V **11**	GA-VIII **13**	GA-IX **14**	GA-IXH$_1$[b)] **17**	GA-IXH$_2$[b)] **18**
1	38.9	38.7	40.4	38.7	38.7	38.8	38.6	38.6	38.7	
2	27.4	25.9	26.0	26.0	26.0	26.0	26.0	25.9	26.0	
3	73.6	82.0	81.4	81.8	81.0	81.8	82.1	81.8	81.7	
4	42.2	42.6	42.4	42.6	42.6	42.7	42.6	42.5	42.6	
5	46.5	47.4	47.6	47.4	47.4	47.4	47.3	47.2	47.3	
6	18.5	18.0	18.2	18.0	18.1	18.1	18.0	18.0	18.0	
7	32.7	32.6	32.7	32.5	32.6	32.5	32.5	32.4	32.5	
8	40.2	40.2	40.4	40.2	40.2	40.3	40.2	40.2	40.2	
9	47.2	47.1	47.3	47.1	47.1	47.1	47.1	47.1	47.1	
10	37.0	36.7	36.8	36.6	36.7	36.7	36.6	36.6	36.7	
11	23.8	23.8	24.0	23.9	23.9	23.9	23.8	23.8	23.9	
12	123.9	123.9	123.3	123.9	123.9	124.2	123.9	123.9	123.9	
13	142.7	142.7	142.8	142.2	142.3	141.5	142.2	142.2	142.3	
14	42.6	43.4	43.5	43.5	43.5	43.5	43.5	43.4	43.5	
15	35.8	35.9	36.1	36.3	36.2	36.8	36.2	35.9	36.2	
16	67.7	68.2	68.4	68.0	68.0	67.0	68.0	68.0	68.0	
17	46.5	46.5	46.8	47.0	47.1	48.0	47.0	47.0	47.1	
18	42.8	42.1	42.8	42.0	42.0	42.7	42.0	41.9	42.0	
19	46.7	46.6	46.8	46.2	46.2	45.9	46.2	46.2	46.0	
20	36.7	36.6	36.7	36.4	36.6	36.7	36.4	36.5	36.6	
21	77.2	77.2	77.2	79.1	79.6	76.6	79.0	79.6	79.6	
22	73.3	73.7	74.1	71.2	71.2	74.6	71.2	71.1	71.2	
23	68.2	64.4	64.6	64.4	64.4	64.4	64.1	64.0	64.1	
24	13.0	13.5	13.5	13.6	13.6	13.6	13.6	13.5	13.6	
25	16.1	16.2	16.2	16.2	16.2	16.2	16.1	16.1	16.2	
26	17.0	17.0	17.1	17.0	17.0	16.9	17.0	16.9	17.0	
27	27.4	27.4	27.4	27.4	27.4	27.5	27.4	27.4	27.4	
28	58.5	58.6	59.1	58.1	58.1	59.9	58.1	58.0	58.1	
29	30.0	30.3	30.3	29.6	29.6	29.2	29.6	29.5	29.6	
30	19.0	18.9	18.9	19.8	19.9	19.8	19.8	19.8	19.9	
GluA										
1′		106.0	106.2	106.1	105.5	106.1	106.3	105.9	105.6	105.6
2′		75.3	75.4	75.4	75.1	75.4	72.1	71.8	74.4	74.2
3′		78.0	77.9	78.1	78.2	78.1	73.8	73.7	85.1	87.4
4′		73.3	73.1	73.4	73.5	73.4	69.6	69.5	71.9	71.4
5′		77.7	77.4	77.8	77.4	77.6	75.2	75.0	76.3	77.0
6′		172.7	170.6	172.9	173.8	172.9	171.5	172.2	172.8	172.6
OMe			51.9							
Second sugar										
1″							97.0	96.8	102.3	105.6
2″							93.8	93.7	71.6	75.2
3″							79.7	79.4	75.4	78.7
4″							69.5	69.3	68.4	71.5
5″							79.4	78.9	78.8	78.0
6″							62.8	62.5	62.3	62.4
Acyl group										
1				176.6	168.2	167.6	176.6	168.5	168.3	
2				42.0	129.7	128.9	42.0	129.6	129.7	
3				27.2	136.4	137.6	27.2	136.8	136.6	
4				12.0	12.4	12.2	12.0	12.4	12.4	
5				17.1	14.1	14.1	17.1	14.2	14.2	
1′						167.8				
2′						128.9				
3′						138.0				
4′						12.2				
5′						14.2				

a) Chemical shift (δ) in C_5D_5N containing a few drops of D_2O with TMS as an internal standard. *b)* $NaBH_4$ reduction product of GA-IX.

bardment mass spectrum (FAB-MS) of **6** exhibited the quasi-molecular ion peak at m/z 683 ($M^+ + 1$) indicating that it is a gymnemagenin glucuronide. DAGA was hydrolyzed with β-glucuronidase to give gymnemagenin (**1**), mp >300 °C, which gave the hexaacetate (**2**), mp 298—300 °C, on usual acetylation. These data and the NMR spectrum of the hexaacetate (**2**) indicated that our genin is identical with Stöcklin's gymnemagenin.[14)]

When gymnemagenin (**1**) was treated with 2,2-dimethoxypropane in acetone under pyridinium p-toluenesulfonate (PPTS) catalysis at room temperature, it gave a mixture of triacetonides, which decomposed rapidly on measurement of the NMR spectrum in $CDCl_3$. Thus, the crude triacetonide was treated with chloroform to convert it into a diacetonide (**3**), which formed colorless prisms of mp 296—298 °C from benzene–hexane and was proved to

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be identical with the diacetonide B described by Stöcklin[14b] on the basis of the spectral data of **3** and the derived diacetate (**4**), mp 296—300 °C.

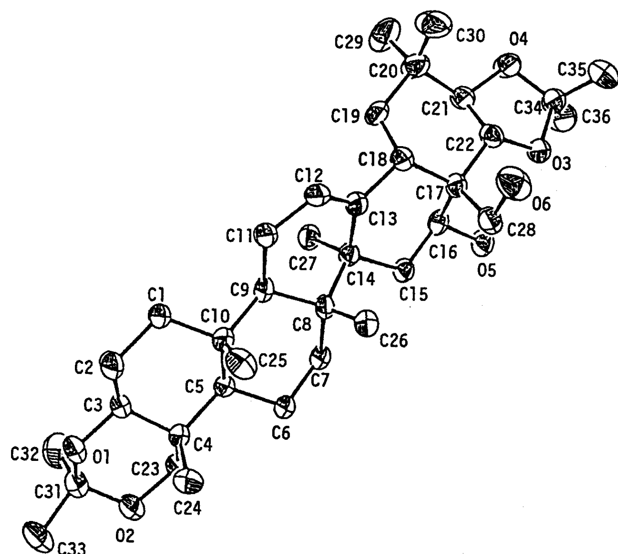

Fig. 1. ORTEP Drawing of Gymnemagenin Diacetonide **3**

A single crystal of this diacetonide was subjected to X-ray crystallographic analysis. The result is shown in Fig. 1, confirming not only the proposed structure of gymnemagenin (**1**) but also the structure of Stöcklin's diacetonide B (3*β*,23;21*β*,22*α*-di-*O*-isopropylidene-16,28-dihydroxyolean-12-ene).

Based on the established structure of gymnemagenin (**1**), the ^{13}C-NMR signals of the gymnemagenin (**1**) and DAGA (**5**) were assigned as shown in Table III. The large glycosylation shifts[21] (+8.4 and +7.8 ppm) at C-3 in both DAGA (**5**) and its methyl ester (**6**) clearly indicated that glucuronic acid is located at C_3-O on the aglycone. Glycosylation shifts at C-23, C-4, and C-2 are negative, slightly positive, and negligible, respectively, supporting this assignment. Thus DAGA (**5**) is gymnemagenin 3-*O*-*β*-D-glucuronide.

Isolation of Gymnemic Acid-III, -IV, -V, -VIII, and -IX Here we describe our independent isolation of gymnemic acids. The procedure is schematically shown in Chart 2, and mainly involves chromatography of the saponin mixture on an ODS column with various solvent systems. Briefly, the crude saponin mixture obtained by Kurihara's procedure[11] was firstly chromatographed on an ODS column with a gradient of MeOH : H_2O (50 : 50→70 : 30→

Table IV. ^{1}H-NMR Data for Gymnemic Acids and DAGA[a]

H	DAGA (**5**)	GA-III (**9**)	GA-IV (**10**)	GA-V (**11**)	GA-VIII (**13**)	GA-IX (**14**)
Genin						
3	4.30 (dd, 11.5, 4.5)	4.32 (dd, 11.5, 4.5)	4.34 (dd, 12.0, 4.5)	4.34 (dd, 11.0, 4.0)	4.28 (dd, 11.0, 4.0)	4.28 (dd, 11.0, 4.0)
12	5.34 (br t)	5.32 (br t)	5.34 (br t)	5.38 (br t)	5.35 (br t)	5.41 (br t)
16	5.05 (dd, 11.5, 5.0)	5.09 (dd, 11.0, 5.0)	5.10 (dd, 11.5, 5.0)	5.04 (dd, 11.0, 5.0)	5.08 (dd, 11.0, 5.0)	5.10 (dd, 11.0, 5.0)
18	3.18 (dd, 14.5, 4.5)	3.15 (dd, 13.5, 3.0)	3.20 (dd, 14.0, 3.5)	3.12 (dd, 13.5, 3.5)	3.17 (dd, 14.0, 3.5)	3.20 (dd, 13.0, 3.5)
21	4.04[b] (d, 10.5)	5.68 (d, 11.0)	5.80 (d, 11.0)	5.83[b] (d, 11.5)	5.69 (d, 11.0)	5.76[c] (d, 11.0)
22	4.86[b] (d, 10.5)	4.95 (d, 11.0)	5.04 (d, 11.0)	6.35[b] (d, 11.5)	4.95 (d, 11.0)	5.00 (d, 11.0)
23	4.33 (d, 11.0)	4.33 (d, 11.0)	4.32 (d, 11.0)	4.34 (d, 11.0)	4.32 (d, 11.0)	4.29 (d, 11.0)
	3.69 (d, 11.0)	3.69 (d, 11.0)	3.68 (d, 11.0)	3.70 (d, 11.0)	3.71 (d, 11.0)	3.68 (d, 11.0)
28	4.71 (d, 10.0)	4.04 (d, 11.0)	4.08 (d, 11.0)	4.03 (d, 11.0)	4.05 (d, 11.0)	4.08 (d, 11.0)
	4.07 (d, 10.0)	4.65 (d, 11.0)	4.71 (d, 11.0)	4.24 (d, 11.0)	4.67 (d, 11.0)	4.69 (d, 11.0)
24	0.96 (s)	0.92 (s)	0.94 (s)	0.95 (s)	0.94 (s)	0.94 (s)
25	0.90 (s)	0.87 (s)	0.89 (s)	0.90 (s)	0.87 (s)	0.87 (s)
26	0.95 (s)	0.91 (s)	0.94 (s)	0.93 (s)	0.94 (s)	0.92 (s)
27	1.35 (s)	1.28 (s)	1.30 (s)	1.29 (s)	1.29 (s)	1.31 (s)
29	1.25 (s)	1.03 (s)	1.04 (s)	1.03 (s)	1.04 (s)	1.03 (s)
30	1.35 (s)	1.28 (s)	1.30 (s)	1.32 (s)	1.29 (s)	1.31 (s)
GluA						
1′	5.25 (d, 7.5)	5.22 (d, 7.5)	5.21 (d, 7.5)	5.23 (d, 7.5)	5.20 (d, 7.2)	5.19 (d, 7.2)
2′	4.12 (dd, 7.5, 8.0)	4.12 (dd, 7.5, 8.0)	4.12 (dd, 7.2, 8.0)	4.14 (dd, 7.5, 8)	4.19 (dd, 7.2, 9.6)	4.18 (dd, 7.2, 9.6)
3′	4.22 (t, 8.0)	4.21 (t, 8.0)	4.22 (t, 8.0)	4.22 (t, 8.0)	4.91 (t, 9.6)	4.91 (t, 9.6)
4′	4.54 (t, 8.0)	4.50 (t, 8.0)	4.46 (t, 8.0)	4.50 (t, 8.0)	5.15 (t, 9.6)	5.10 (t, 9.6)
5′	4.57 (d, 8.0)	4.53 (d, 8.0)	4.49 (d, 8.0)	4.52 (d, 8.0)	4.51 (d, 9.6)	4.51 (d, 9.6)
Second sugar						
1″					5.28 (s)	5.26 (s)
2″					—	—
3″					4.22 (d, 9.6)	4.21 (d, 9.6)
4″					4.39 (t, 9.6)	4.41 (t, 9.6)
5″					4.02 (dd, 4.0, 9.6)	4.02 (dd, 4.0, 9.6)
6″					4.60 (br d, 11.2)	4.59 (br d, 11.2)
					4.33 (dd, 4.0, 11.2)	4.29 (dd, 4.0, 11.2)
Acyl group						
		0.99 (t, 7.0)	1.66 (d, 7.0)	1.48 (d, 7.0)	0.99 (t, 7.0)	1.66 (d, 7.0)
		1.23 (d, 7.0)	1.88 (s)	1.62 (d, 7.0)	1.23 (t, 7.0)	1.89 (s)
		1.52 (m)	7.01 (q, 7.0)	1.84 (s)	1.52 (m)	7.02 (q, 7.0)
		1.82 (m)		1.89 (s)	1.82 (m)	
		2.56 (m)		7.03 (q, 7.0)	2.56 (m)	

a) Chemical shift (*δ*) in C_5D_6N containing a few drops of D_2O with TMS as an internal standard. The data in parentheses are multiplicities and coupling constants. *b*) Those data were confirmed by ^{1}H–^{13}C COSY experiments. The assignments of H-21 and H-22 by Yoshikawa *et al.*[16] must be revised. *c*) This peak is overlapped with the HDO signal.

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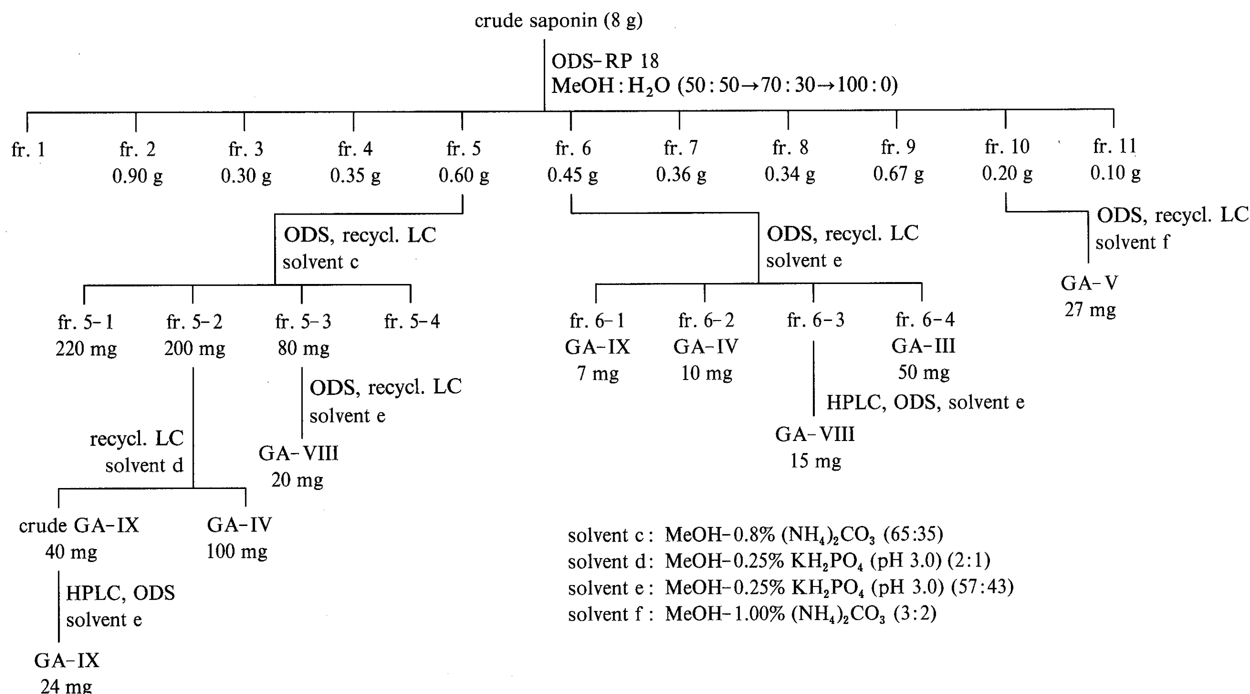

Chart 2

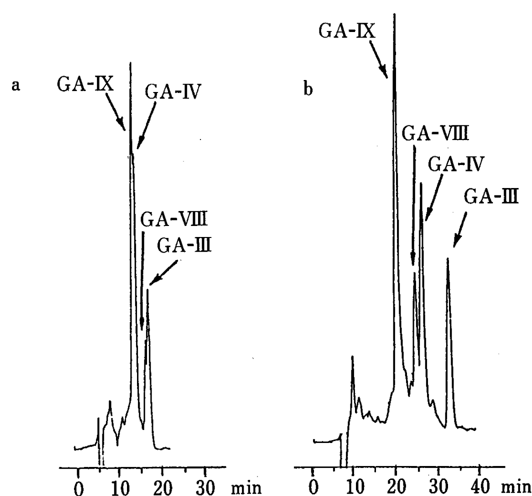

Fig. 2. HPLC Behaviors of GA-III, -IV, -VIII, and -IX

Column, TSK gel ODS-120T (4.6 × 250 mm); detection, refractive index; flow rate, 0.5 ml/min. Solvent: a) MeOH–0.8% $(NH_4)_2CO_3$ (pH 8) (62 : 38); b) MeOH–0.25% KH_2PO_4 (pH 3) (62 : 38).

100 : 0) to obtain 11 fractions. The diacyl derivative (GA-V) was eluted in the late fractions with this solvent system. Fractions 5 and 6 were then chromatographed with the solvent containing ammonium carbonate buffer (pH 8)[22] to give mixtures of GA-IV and GA-IX, and GA-III and GA-VIII, respectively. The mixture of GA-III and -VIII and that of GA-IV and -IX were separated into each component by chromatography with the solvents containing KH_2PO_4 (pH 3) buffer. Other solvent systems examined did not give good separation. Thus, we isolated five gymnemic acids (GA-III, IV, V, VIII, and IX),[19] of which GA-III, IV, and V were identical with Yoshikawa's gymnemic acids GA-III, IV, and V,[16] respectively, and GA-VIII and IX were new compounds.

Structures of GA-III, IV, and V Comparisons of the ^1H- and ^{13}C-NMR spectra of GA-III and GA-IV revealed that they are derivatives of DAGA **5** and that they differ only in the acyl group, which is 2-methylbutyryl in GA-III and tigloyl in GA-IV. The acyl group is at the same position in both compounds, *i.e.*, at C_{21}-OH in the aglycone, for the reasons described below.

For determination of the position of the acyl group, ^1H–^1H and ^1H–^{13}C chemical shift correlation spectra (COSY) of GA-III were examined in detail. In the ^1H-NMR spectra, H-21 and H-22 appeared at δ 5.68 and 4.95 for GA-III (and at δ 5.80 and 5.04 for GA-IV). These protons were directly attached to the carbons at δ 79.1 and 71.2 for GA-III (and at δ 79.6 and 71.2 for GA-IV), one of which is shifted downfield and the other upfield compared to the corresponding carbons in DAGA (δ 77.2 and 73.7). Therefore, if we can assign these carbon signals correctly, the position of the acyl group will be determined by the acylation shift rule.[23]

The carbon signal attributable to C-18 appeared at δ 42.0 (d), which is correlated with the proton at δ 3.15 (1H, dd). Irradiation of this proton (H-18) produced nuclear Overhauser effect (NOE) on the olefinic proton (H-12, δ 5.32) with 5.7% enhancement of the intensity, as well as the proton at δ 4.92 (1H, d) with 6.2% enhancement, indicating that the latter proton is diaxially oriented to H-18, *i.e.*, H-22, which is again correlated to the carbon signal at δ 71.2. Therefore, the carbon signals at δ 79.1 and 71.2 are those of C-21 and C-22, respectively. They appear at δ 79.6 and 71.2 in GA-IV. Thus the acylation shifts of these carbons are +1.9 ppm for C-21 and −2.4 ppm for C-22 in GA-III, and +2.4 ppm for C-21 and −2.1 ppm for C-22 in GA-IV, proving that the acyl group is at C_{21}-O in both compounds. Hence, GA-III is 21-*O*-2-methylbutyryl-DAGA (**7**) and GA-IV is 21-*O*-tigloyl-DAGA (**8**). These structures are in agreement with those assigned for GA-III and GA-IV by Yoshikawa *et al.*[16a] In fact, the ^1H- and ^{13}C-NMR spectra of our GA-III were identical with those

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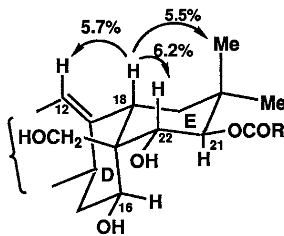

Chart 3. Observed NOE in GA-III

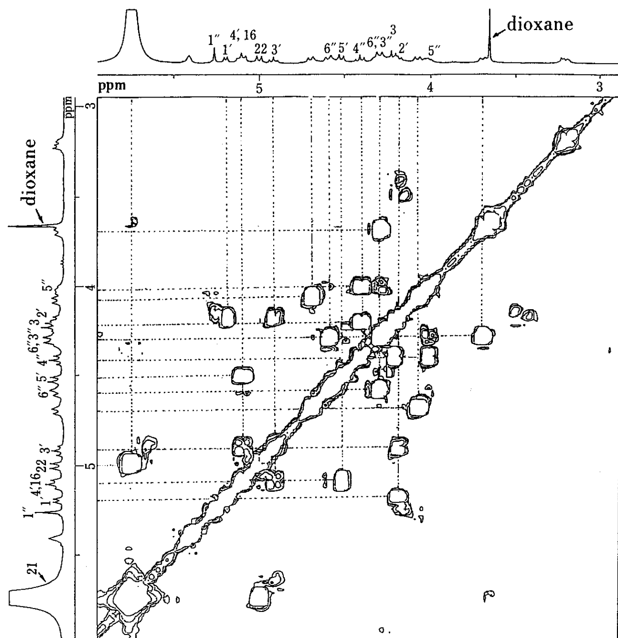

Fig. 3. ^1H–^1H COSY Spectrum of GA-IX

The ^1H signal at δ 5.76 (H-21) is overlapped with the large HDO signal in this solvent system.

of GA-III kindly provided by Prof. Arihara.

GA-V (**9**) bears two tigloyl groups on DAGA. The protons at C-21 and C-22 showed significant downfield shifts (H-21: δ 4.04→5.83 and H-22: δ 4.86→6.35), thus indicating that both C_{21}-OH and C_{22}-OH are acylated with tigloyl groups. This assignment agrees with Yoshikawa's results.[16b)]

Structures of New Gymnemic Acids GA-VIII (13) and GA-IX (14) The high resolution FAB-MS (HR-FAB-MS) (+NaCl) of GA-VIII and GA-IX indicated their formulae to be $C_{47}H_{74}O_{18}$ and $C_{47}H_{72}O_{18}$ from the $[M+Na]^+$ peaks at m/z 949.4818 and 947.4681, respectively. The infrared (IR) spectra of both compounds showed a carbonyl absorption at 1730 cm^{-1}. The ^1H- and ^{13}C-NMR spectra indicated that GA-VIII and GA-IX contain a 2-methylbutyryl group and a tigloyl group, respectively. This was supported by the intense peaks at m/z 765 and 763 in the negative ion FAB-MS, which corresponded to DAGA acylated by the above acyl groups, respectively.

The sugar portions of GA-VIII and GA-IX should be identical, because they gave similar signal patterns in the ^1H- and ^{13}C-NMR spectra for the sugar region, and they appear to consist of glucuronic acid and another hexose as judged from their formulae. Thus, GA-VIII and GA-IX only differ in the acyl groups, which are supposed to be at C-21 from the similarity of the spectra with those of GA-III and GA-IV, respectively, suggesting that the former compounds are derivatives of GA-III and GA-IV with the same hexose moiety.

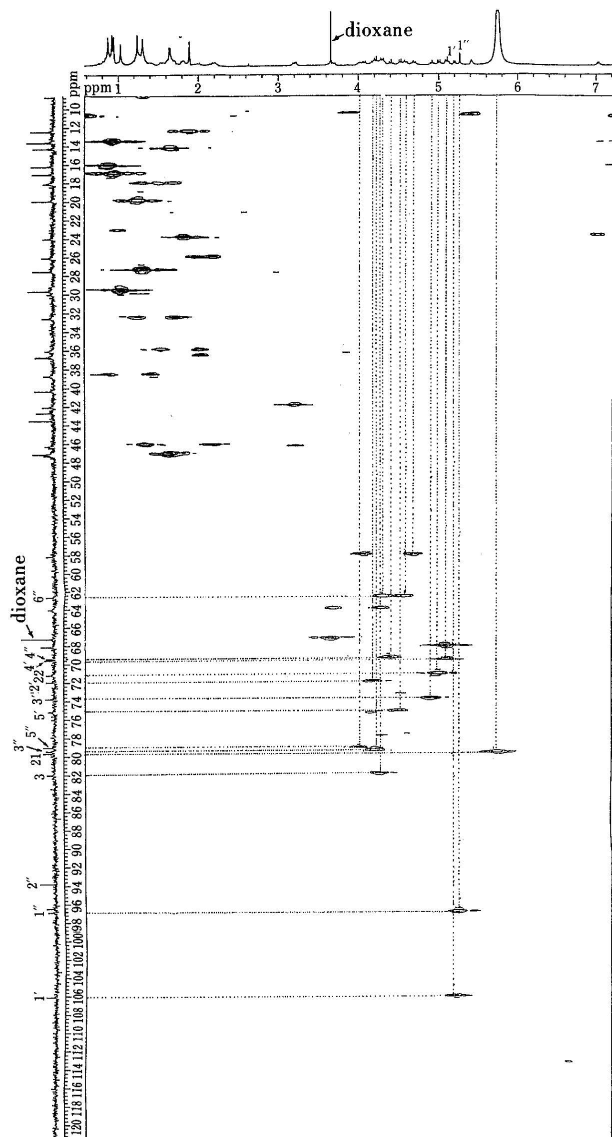

Fig. 4. ^1H–^{13}C COSY Spectrum of GA-IX.

Inspections of the 2D-NMR spectra (^1H–^1H and ^1H–^{13}C COSY, Figs. 4 and 5) of GA-IX (**14**) revealed that two different series of hexose signals were present in the molecule, which corresponded to 12 peaks in the ^{13}C-NMR, including the signal at δ 172.2 assignable to COOH. In the first sugar moiety, the proton at δ 5.19 (d, J=7.2 Hz) is obviously the anomeric proton, which is correlated to a carbon at δ 105.9. Since H-5 (δ 4.51, d, J=9.6 Hz) appeared as a doublet and connectivities between only five protons were found in this portion, we assumed that the COOH group (δ 172.2) is connected to C-5. This sugar portion corresponds to a glucuronic acid residue which is a β-isomer because the coupling constant of the anomeric proton is 7.2 Hz and the anomeric carbon signal appeared at δ 105.9.

For the second sugar moiety, the connectivities between five protons were revealed; the protons at δ 4.59 and δ 4.29 are connected to the same carbon (δ 62.5, t), showing that

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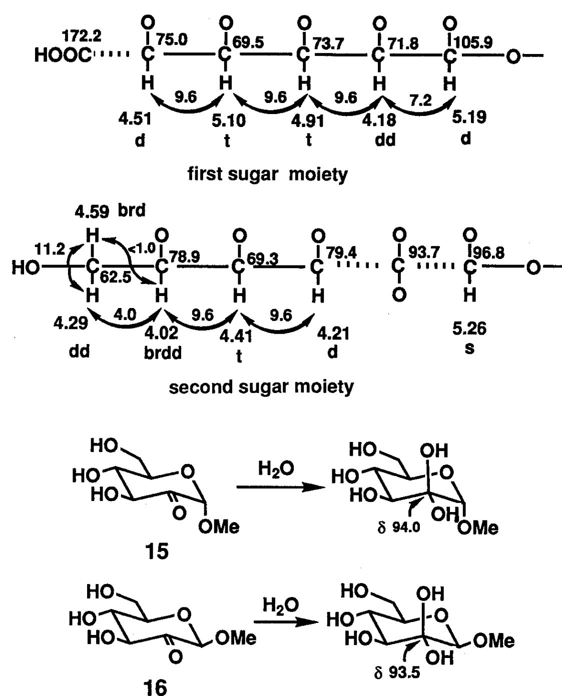

Chart 4. Connectivity of Sugar Portions in GA-IX

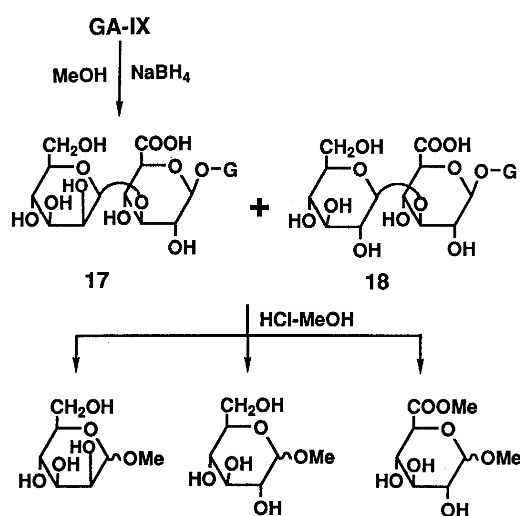

Chart 5

this group is –CH_2OH. The anomeric proton appeared as a singlet at δ 5.26, which is correlated with the carbon at δ 96.8 (d), indicating that no proton is attached to C-2. A singlet carbon signal at δ 93.7 must correspond to this carbon. This chemical shift agrees with the shift value found in a hydrate form of an oxo-glycoside. Actually, methyl α-D-*arabino*-hexopyranosid-2-ulose (**15**) and methyl β-D-*arabino*-hexopyranosid-2-ulose (**16**) gave signals at δ 94.0 and δ 93.5 for C-2, respectively, in D_2O without showing any carbonyl absorption.[24] We therefore presumed that GA-IX is the derivative of GA-IV connected with a 2-oxo-hexose which is forming an intramolecular hemiacetal with another hydroxy group (see below).

This idea was supported by the observation that GA-IX, when kept in pyridine-d_5 for a long time, gradually liberated GA-IV. Such a property is characteristic of 2-oxo-glycosides.[25]

In order to identify conclusively the second sugar moiety, we carried out sodium borohydride reduction of GA-IX in methanol and analyzed the resulting product by ^{13}C-NMR, because it is known[26] that oxo-glycosides behave as ketones in hydride reduction, even if they form hydrates or dimeric hemiacetals. Although the product was a mixture of two compounds (major : minor = 3 : 1; the ratio was determined from the result of methanolysis, see below), the major constituent showed 12 peaks (including COOH) due to the sugar moiety indicating that it is a disaccharide. The terminal sugar moiety of this compound was now revealed to be a β-D-mannoside (**17**), since the six signals assignable to this moiety were in good agreement with those of methyl β-D-mannopyranoside. The ^{13}C-NMR data of the minor product were similar with those of the major product except for the terminal sugar portion, which was assigned as a β-D-glucoside (**18**).

The above assignments were confirmed by methanolysis of the reduction mixture. The sugar fraction of the product was a mixture of methyl (α and β) D-glucoside, methyl (α and β) D-mannoside, and methyl glucuronide methyl ester in the ratio of 1 : 3 : 4 as revealed by gas chromatography of the trimethylsilyl derivatives. These results proved that GA-IX is the derivative of GA-IV connected with 2-oxo-glucose.

Comparisons of the ^{13}C-NMR data of the mannoside (**17**) with GA-IV showed a large positive glycosylation shift at C-3′ (+7.0 ppm) and negative shifts at C-2′ and C-4′ of the glucuronide portion, indicating that the mannose moiety is attached at O-3′ of the glucuronide. Thus, we can conclude that the major reduction product is GA-IV 3′-*O*-β-D-mannopyranoside (**17**) and the minor product is GA-IV 3′-*O*-β-D-glucopyranoside (**18**). In fact, the carbon chemical shifts of **18** are identical with those of GA-VI reported by Yoshikawa *et al.*[16b] Therefore GA-IX is concluded to be 3′-*O*-β-D-*arabino*-2-hexulopyranosyl GA-IV (**14**). GA-VIII must be the corresponding derivative of GA-III (**13**).

Stereostructures of GA-VIII and GA-IX As mentioned above, the oxo group in the 2-oxohexose moiety is forming a hydrate or a hemiacetal, since the corresponding carbon did not show any signal at the δ 190—210 region in the ^{13}C-NMR spectra, but instead exhibited a signal at δ 93.7 in GA-IX and at δ 93.8 in GA-VIII. This was supposed to indicate an intramolecular hemiacetal forming a dioxane ring, since the glucuronide carbons in GA-IX (and GA-VIII) appeared at unusually higher field than those expected for a glucuronide carrying a hexose at O-3′, while the corresponding carbons in the reduction product (**17**) showed a normal glycosylation shift. These suggested that one of the hydroxyls at C′-2 or C′-4 is forming a cyclic structure.

The conformational stability of the dioxane ring between the two sugar portions was calculated by MM2 for the simplest model, 1,4-dioxane: the result revealed that the $^0C_0$ conformation is the most stable, while twist and $_2B_5$ forms have larger steric energies ($\Delta\Delta H$ = 5.4 and > 6 kcal/mol, respectively). Taking account of these data, we consider that the structure of GA-VIII is **13a** or **13b**, which corresponds to the hemiacetal linkage with O-4′ or O-2′ in an axial or equatorial mode, respectively; any other connections of O-2″ to O-4′ or O-2′ produce boat conformations of $_2B_5$ type for the dioxane ring.

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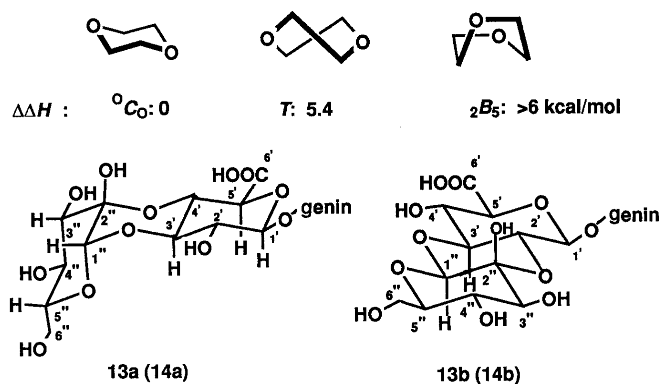

Chart 6

Then, NOE experiments on GA-VIII were carried out. On irradiation of H-1″, the signal intensities of H-3″ and H-5″ were enhanced by 6.2% and 4.1%, respectively. When H-3′ was irradiated, the intensities of only H-1′ and H-5′ were enhanced by 8.7% and 6.0%, respectively. Those results indicate that the glucuronide and oxo-sugar moieties are in *C1* conformations, supporting the assignment based on the coupling constants in the ^{1}H-NMR data. However, no NOE between H-1″ and H-3′ was observed on irradiation of either H-1″ or H-3′. If GA-VIII had the structure **13b**, obvious NOE would be expected between these protons because they are diaxially oriented. For these reasons, we conclude that GA-VIII and GA-IX have the stereostructures **13a** and **14a**, respectively.

This conclusion does not necessarily mean that GA-VIII and GA-IX exist with this form in the plant, since hemiacetals or hydrates of oxo-glycosides equilibrate with the oxo-forms depending on the solvent.[24] Therefore, whatever the forms in which they exist in the plant, the compounds isolated through solvent extraction and chromatography should have the most stable conformation for the hemiacetal moiety of the oxo-glycoside portion. Such considerations may be generally applicable to glycosides containing an oxo-sugar moiety.

Experimental

Unless otherwise stated, the following procedures were adopted. Melting points were determined on a Yanaco micro hot stage melting point apparatus and were not corrected. IR spectra were taken in KBr disks and data are given in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra were taken in pyridine-d_5 containing a few drops of D_2O with tetramethylsilane as an internal standard on a JEOL GX-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) and the chemical shifts are given in δ values. ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. Mass spectra (MS) were measured with a Hitachi M-80 machine. Positive and negative ion FAB-MS were recorded on a JEOL GM-HX 110 spectrometer. Optical rotations were measured on a JASCO DIP 181. Column chromatography was performed on silica gel (Fuji-Davison BW-820 MH) or ODS (Merck Lichroprep RP-18). High performance liquid chromatograph (HPLC) was performed on TSK-gel ODS-120T using a Toso CCPDF pump with an RI-8000 refractive index (RI) detector. Recycling LC was carried out on Inertsil (Prep-ODS) using an LC-980 apparatus (Nihon Bunseki Kogyo) with an RI detector. For TLC, Merck precoated plates GF_{254} were used and spots were detected by spraying 5% H_2SO_4 and heating at 100 °C until coloration took place.

Deacylgymnemic Acid (DAGA) (5) The crude saponin mixture obtained by water extraction of dried gymnema leaves (see below) was purified by extraction with diethyl carbonate and chromatography over a DEAE-Sephadex A-25 column as stated by Kurihara.[11] The saponin mixture thus obtained was hydrolyzed with 3% KOH–MeOH under reflux for 15 min. The mixture was concentrated to dryness and the residue was dissolved in water. This solution was acidified with 2 N H_2SO_4. The precipitate was collected by centrifugation and chromatographed on a silica gel column with AcOEt–MeOH (9 : 2) to yield DAGA (**5**) as a colorless powder, mp 230—235 °C. FAB-MS (pos.) *m/z*: 683 ($M^+ +1$). ^{1}H- and ^{13}C-NMR: see Tables IV and III.

Deacylgymnemic Acid Methyl Ester (6) A solution of DAGA (50 mg) in MeOH (25 ml) was treated with ethereal diazomethane for 1 h at room temperature. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (9 : 1) as an eluant to yield **6** (44 mg). ^{1}H-NMR: 5.34 (1H, br t, H-12), 5.12 (1H, d, J=7.5 Hz, H-1′), 5.03 (1H, dd, J=11.0, 5.5 Hz, H-16), 4.78 (1H, d, J=10.5 Hz, H-22), 4.66, 4.04 (each 1H, d, J=11.0 Hz, 17β-CH_2OH), 4.42 (1H, d, J=8.0 Hz, H-5′), 4.37 (1H, t, J=8.0 Hz, H-4′), 4.26, 3.67 (each 1H, d, J=11.0 Hz, 4α-CH_2OH), 4.23 (1H, dd, J=12.0, 4.5 Hz, H-3), 4.11 (1H, t, J=8.0 Hz, H-3′), 4.03 (1H, dd, J=8.0, 7.5 Hz, H-2′), 4.01 (1H, d, J=10.5 Hz, H-21), 3.69 (3H, s, OMe), 1.28, 1.25, 1.22, 1.00, 0.97 × 2 (each 3H, s, Me). ^{13}C-NMR: see Table III.

Gymnemagenin (1) A mixture of DAGA (100 mg) and β-glucuronidase (Sigma, Type H-2, crude solution from *Helix pomatia*, 120000 units/ml, 0.2 ml) in 0.2 M NaOAc–AcOH buffer (pH 4.8, 50 ml), was incubated at 37 °C for 24 h, and then the mixture was extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4, then concentrated to dryness, and the residue was chromatographed on silica gel with $CHCl_3$–MeOH to give gymnemagenin (**1**) (70 mg) as colorless needles, mp >300 °C (lit. mp 328—335 °C).[14a] $[\alpha]_D^{20}$ +53.9 (c=0.75, MeOH). ^{1}H-NMR: 5.38 (1H, br t, H-12), 5.03 (1H, dd, J=11.0, 5.5 Hz, H-16), 4.86 (1H, d, J=11.0 Hz, H-22), 4.72, 4.09 (1H, d, J=11.0 Hz, 17β-CH_2OH), 4.18 (1H, dd, J=11.0, 5.5 Hz, H-3), 4.14, 3.69 (1H, J=11.0 Hz, 4α-CH_2OH), 4.05 (1H, d, J=11.0 Hz, H-21), 3.20 (1H, dd, J=14.5, 4.5 Hz, H-18), 1.33, 1.30, 1.26, 1.05, 1.00, 0.96 (each 3H, s, Me). MS *m/z*: 506 (M^+, 0.3%), 488 ($M^+ -18$, 18%), 215 (100%). ^{13}C-NMR: see Table III.

Gymnemagenin (15 mg) was treated with acetic anhydride–pyridine under *N,N*-dimethylaminopyridine (DMAP, 2 mg) catalysis for 10 h at room temperature. The mixture was poured into water and extracted with ether. The ethereal extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. Chromatography of the residue on silica gel with benzene–AcOEt gave the hexaacetate (**2**) (15 mg) as a colorless powder, mp 296—300 °C (lit. mp 290—291 °C).[14a] ^{1}H-NMR ($CDCl_3$, 100 MHz): 5.80 (1H, dd, J=11.0, 5.5 Hz, H-16), 5.36 (1H, d, J=11.0 Hz, H-22), 5.30 (1H, br t, H-12), 5.07 (1H, d, J=11.0 Hz, H-21), 4.68 (1H, dd, J=10.0, 5.5 Hz, H-3), 4.21, 3.88 (each 1H, d, J=11.0 Hz, CH_2OAc), 3.81, 3.61 (each 1H, d, J=12.0 Hz, CH_2OAc), 2.02, 1.99, 1.96 × 2, 1.92, 1.86 (each 3H, s, OAc), 1.22, 1.00, 0.91 × 2, 0.87, 0.77 (each 3H, s, Me). MS *m/z*: 758 (M^+, 0.17%).

3β,23;21β,22α-Di-*O*-isopropylidenegymnemagenin (3) A mixture of gymnemagenin (**1**) (55 mg), 2,2-dimethoxypropane (8 ml), and PPTS (50 mg) in acetone (40 ml) was stirred for 2 h at room temperature. The mixture was concentrated to dryness under reduced pressure and the residue in $CHCl_3$ (10 ml) was stirred for 30 min at room temperature. The mixture was poured into a column of silica gel and eluted with benzene–AcOEt (4 : 1) to give the diacetonide **3** (20 mg), which was crystallized from hexane–benzene as colorless prisms, mp 296—298 °C (lit. diacetonide B, mp 280—281 °C).[14b] ^{1}H-NMR ($CDCl_3$, 100 MHz): 5.31 (1H, br t, H-12), 4.50 (1H, dd, J=11.0, 5.0 Hz, H-16), 4.04, 3.87 (each 1H, d, J=12.0 Hz, 17β-CH_2OH), 3.91 (1H, d, J=10.0 Hz, H-22), 3.64 (1H, d, J=10.0 Hz, H-21), 3.57, 3.41 (each 1H, d, J=11.0 Hz, 4α-CH_2O–), 3.48 (1H, dd, J=10.0, 6.0 Hz, H-3), 1.56 (6H, s, O_2CMe_2), 1.43 (3H, s, Me), 1.40 (6H, s, O_2CMe_2), 1.20, 1.00, 0.94 × 3 (each 3H, s, Me). MS *m/z*: 576 ($M^+ -15$, 26%), 568 ($M^+ -18$, 100%).

This compound was treated with acetic anhydride–pyridine under DMAP catalysis for 20 h at room temperature to give the diacetate **4** as a colorless powder, mp 237—240 °C (lit. mp 242—245 °C).[14b] ^{1}H-NMR ($CDCl_3$, 100 MHz): 5.77 (1H, dd, J=12.0, 5.5 Hz, H-16), 4.28 (2H, s, 17β-CH_2OAc), 5.31 (1H, br t, H-12), 3.77 (1H, d, J=10.0 Hz, H-22), 3.60 (1H, d, J=10.0 Hz, H-21), 3.54, 3.43 (each 1H, d, J=11.0 Hz, 4α-CH_2O–), 3.48 (1H, dd, J=10.0, 6.0 Hz, H-3), 2.00, 2.08 (each 3H, s, OAc), 1.64 (6H, s, O_2CMe_2), 1.45, 1.42 (each 3H, s, O_2CMe_2), 1.33, 1.31, 1.26, 1.05, 1.00, 0.97 (each 3H, s, Me).

X-Ray Crystallographic Analysis of the Di-*O*-isopropylidene Derivative 3 Reflection data were collected on a Rigaku AFC-5R four-circle diffractometer controlled by the MSC/AFC program package, using Mo$K\alpha$ radiation monochromated by a graphite monochromator, in the 2θ–ω scan mode. Of the total of 3365 reflections, 2616 with intensity above the 3σ (I) level were used for the structure determination. The structure was solved

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Table V. Positional Parameters for Gymnemagenin Diacetonide (**3**)

Atom	x	y	z	B_{eq}
O1	0.4518 (2)	0.3875 (2)	0.271 (1)	4.4 (2)
O2	0.5282 (2)	0.4369 (2)	0.0905 (8)	4.2 (2)
O3	0.2123 (2)	0.9255 (2)	0.1726 (7)	3.4 (2)
O4	0.1134 (2)	0.9121 (2)	0.0749 (8)	4.0 (2)
O5	0.3045 (2)	0.8611 (2)	0.0100 (7)	3.4 (2)
O6	0.2449 (2)	0.8566 (2)	0.6262 (8)	5.0 (3)
C1	0.3266 (2)	0.4912 (2)	0.409 (1)	3.4 (3)
C2	0.3685 (3)	0.4364 (3)	0.424 (1)	4.4 (3)
C3	0.4153 (3)	0.4399 (3)	0.263 (1)	3.4 (3)
C4	0.4553 (2)	0.4950 (2)	0.278 (1)	2.9 (2)
C5	0.4117 (2)	0.5490 (2)	0.262 (1)	2.6 (2)
C6	0.4443 (2)	0.6088 (2)	0.241 (1)	3.4 (3)
C7	0.3997 (3)	0.6566 (3)	0.170 (1)	3.0 (3)
C8	0.3447 (2)	0.6633 (2)	0.303 (1)	2.7 (2)
C9	0.3163 (3)	0.6014 (2)	0.348 (1)	2.6 (2)
C10	0.3605 (3)	0.5510 (2)	0.421 (1)	2.7 (2)
C11	0.2600 (3)	0.6069 (3)	0.478 (1)	3.2 (3)
C12	0.2274 (3)	0.6654 (2)	0.462 (1)	3.0 (3)
C13	0.2421 (2)	0.7098 (2)	0.342 (1)	2.7 (2)
C14	0.2945 (2)	0.7019 (2)	0.1965 (9)	2.4 (2)
C15	0.3212 (2)	0.7622 (2)	0.123 (1)	2.6 (2)
C16	0.2744 (3)	0.8095 (2)	0.090 (1)	2.5 (2)
C17	0.2378 (2)	0.8222 (2)	0.278 (1)	2.5 (2)
C18	0.2017 (3)	0.7647 (2)	0.335 (1)	2.9 (3)
C19	0.1482 (3)	0.7528 (2)	0.193 (1)	3.9 (3)
C20	0.1027 (3)	0.8041 (3)	0.162 (1)	4.6 (3)
C21	0.1429 (3)	0.8556 (3)	0.095 (1)	3.6 (3)
C22	0.1899 (2)	0.8695 (2)	0.252 (1)	2.7 (3)
C23	0.4943 (3)	0.4910 (3)	0.089 (1)	3.7 (3)
C24	0.4962 (3)	0.4950 (3)	0.462 (1)	4.3 (3)
C25	0.3827 (3)	0.5598 (3)	0.639 (1)	3.8 (3)
C26	0.3651 (2)	0.6945 (2)	0.501 (1)	3.3 (3)
C27	0.2701 (3)	0.6704 (2)	0.004 (1)	3.0 (3)
C28	0.2793 (3)	0.8401 (3)	0.454 (1)	3.1 (3)
C29	0.0597 (3)	0.7863 (3)	−0.011 (2)	7.3 (5)
C30	0.0665 (3)	0.8178 (4)	0.352 (2)	6.6 (5)
C31	0.4933 (3)	0.3836 (3)	0.107 (1)	4.5 (3)
C32	0.4628 (4)	0.3690 (4)	−0.091 (2)	6.1 (5)
C33	0.5390 (3)	0.3367 (3)	0.171 (2)	5.8 (4)
C34	0.1597 (3)	0.9568 (3)	0.093 (1)	3.7 (3)
C35	0.1403 (3)	1.0028 (3)	0.242 (1)	5.0 (4)
C36	0.1755 (3)	0.9810 (3)	−0.108 (1)	5.0 (4)

by MITHRIL[27] and refined by a full-matrix least-squares method using anisotropic temperature factors for non-hydrogen atoms. Positional parameters and the ORTEP drawing of the molecule are given in Table V and Fig. 1, respectively.

Crystal data: $C_{36}H_{58}O_6$, $M_r=586.85$, orthorhombic, $a=22.473$ (3), $b=22.473$ (3), $c=6.600$ (2) Å, $D_c=1.18$ g/cm^{-3}, $Z=4$. Space group, $P2_12_12_1$. $R=0.076$.

Isolation of Gymnemic Acid-III (9), -IV (10), -V (11), -VIII (13), and -IX (14) Dried leaves of *G. sylvestre* (1.5 kg) were extracted (5 l × 5) with water at 60 °C for about 5 h each time. The pH of the combined extracts was adjusted to 3.0 with 2 N H_2SO_4. The precipitate was collected by centrifugation. The precipitate was dissolved in ethanol (1 l) and the insoluble material was removed by centrifugation. The volume of the supernatant was decreased to 0.5 l by evaporation under reduced pressure and acetone (1 l) was added. The precipitate that appeared was removed by centrifugation and the supernatant was concentrated to dryness under reduced pressure to give crude saponins (10.7 g). The crude saponin (8 g) was chromatographed on an ODS column (5 × 25 cm) using MeOH–H_2O 1 : 1→7 : 3→1 : 0 to obtain 11 fractions (frs. 1—11) (see Chart 2). Fraction 5 (0.6 g) was separated by recycling LC using MeOH: 0.8% $(NH_4)_2CO_3$ (pH 8) (65 : 35) as a mobile phase to give four fractions (fr. 5-1—5-4). Fraction 5-2 was again separated by recycling LC using MeOH: 0.25% KH_2PO_4 (pH 3) (2 : 1) to give GA-IV (**6**) (100 mg) and crude GA-IX (40 mg). The crude GA-IX was finally purified by preparative HPLC on an ODS column using MeOH: 0.25% KH_2PO_4 (pH 3) (57 : 43) as a mobile phase to yield pure GA-IX (**14**) (24 mg). Fraction 5-3 was chromatographed on an ODS column by recycling LC using MeOH: 0.25% KH_2PO_4 (pH 3) (57 : 43) as a mobile phase to give GA-VIII (**13**) (20 mg). Fraction 6 was separated on an ODS column by recycling LC followed by preparative HPLC using MeOH: 0.25% KH_2PO_4 (pH 3) (57 : 43) as a mobile phase to give GA-IX (**14**) (7 mg), GA-IV (**10**) (10 mg), GA-III (**9**) (50 mg), and GA-VIII (**13**) (15 mg). Fraction 10 was separated on an ODS column by recycling LC using MeOH: 1.0% $(NH_4)_2CO_3$ (3 : 2) as a mobile phase to give GA-V (**11**) (27 mg).

Gymnemic Acid-III (**9**): Colorless powder, mp 219—221 °C, $[\alpha]_D^{20}$ +9.6° (*c*=0.39, MeOH). IR: 3400, 1715. (lit. mp 218—219 °C, $[\alpha]_D$ +7.6°).[16a] ^{1}H-NMR: see Table IV. ^{13}C-NMR: see Table III.

Gymnemic Acid-IV (**10**): Colorless powder, mp 229—231 °C, $[\alpha]_D^{20}$ +7.4° (*c*=0.21, MeOH). IR: 3400, 1700. (lit. mp 210—221 °C, $[\alpha]_D$ +8.8°).[16a] ^{1}H-NMR: see Table IV. ^{13}C-NMR: see Table III.

Gymnemic Acid-V (**11**): Colorless powder, mp 214—216 °C, $[\alpha]_D^{20}$ +3.3° (*c*=0.30, MeOH). IR: 3400, 1700. (lit. mp 202—203 °C, $[\alpha]_D$ +2.2°).[16b] ^{1}H-NMR: see Table IV. ^{13}C-NMR: see Table III.

Gymnemic Acid-VIII (**13**): Colorless powder, mp 218—220 °C, $[\alpha]_D^{20}$ +17.3°C (*c*=0.74, MeOH). IR: 3450, 1730. FAB-MS (neg.) *m/z*: 925 (M^- −1, 6%), 765 (55%). HR-FAB-MS (pos., +NaCl) *m/z*: 949.4818 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{18}Na$ 949.4773). ^{1}H-NMR: see Table IV. ^{13}C-NMR: see Table III.

Gymnemic Acid-IX (**14**): Colorless powder, mp 222—224 °C $[\alpha]_D^{20}$ +11.4° (*c*=0.70, MeOH). IR: 3400, 1730, 1700. FAB-MS (neg.) *m/z*: 923 (M^- −1, 7%), 763 (33%), 183 (84%). HR-FAB-MS (pos., +NaCl) *m/z*: 947.4681 $[M+Na]^+$ (Calcd for $C_{47}H_{72}O_{18}Na$ 947.4617). ^{1}H-NMR: see Table IV. ^{13}C-NMR: see Table III.

Reduction of GA-IX with $NaBH_4$ A mixture of GA-IX (45 mg) and $NaBH_4$ (90 mg) in MeOH (7 ml) was stirred for 1.5 h at room temperature. The mixture was acidified with 1 N HCl to pH 3.0 and passed through a Diaion HP-20 column. The column was washed with water until the eluate became neutral, and then eluted with MeOH to give the product (26 mg), which was purified by chromatography on an ODS column with MeOH–0.25% KH_2PO_4 buffer (62 : 38). The eluate was neutralized with 5% NH_4OH and passed through a column of Diaion HP-20 to remove inorganic salts. After washing of the column with water, It was eluted with MeOH to give the reduction product (12 mg). The ^{13}C-NMR spectrum (Table III) of this product showed that it is a mixture of two compounds.

Methanolysis of the Reduction Product The above reduction product was heated in 6% HCl–MeOH at 80 °C for 1.5 h, and then concentrated to dryness. The residue was dissolved in pyridine and silylated with hexamethyldisilazane and trimethylsilyl chloride for 10 min at room temperature. The mixture was centrifuged and the supernatant was directly injected into a GC column. GC conditions: column, 1.5% OV-1 on Shimalite W, 2.6 mm i.d. × 2 m; detector, FID; carrier gas, N_2; flow rate, 60 ml/min; column temperature, 155 °C. Retention time (min): 5.4 (Me GluA-Me), 6.7 (Me α-D-Man and Me GluA-Me), 7.7 (Me β-D-Man), 10.8 (Me α-D-Glc), 12.0 (Me GluA-Me and Me β-D-Glc).

Acknowledgements We thank Nichibai Trading Co., Ltd. for supplying us gymnema leaves. We thank Professor K. Shimada, this Faculty, for advice on HPLC analyses, Professors M. Suzuki, and Y. Mori, Meijo University, for the measurement of FAB-MS, and Professor S. Arihara, Tokushima-Bunri University, for the spectra of GA-III.

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