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TRITERPENOID SAPONINS FROM *GYMNEMA SYLVESTRE*

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Key Word Index—*Gymnema sylvestre*; Asclepiadaceae; gymnemasins A, B, C and D; gymnemanol; triterpenes; triterpenoid saponins.

Abstract—Besides six known gymnemic acids, four new tritepenoid saponins, gymnemasins A, B, C and D, isolated from the leaves of *Gymnema sylvestre*, were identified as 3-*O*-[*β*-D-glucopyranosyl(1 → 3)-*β*-D-glucuronopyranosyl]-22-*O*-tigloyl-gymnemanol, 3-*O*-[*β*-D-glucopyranosyl (1 → 3)-*β*-D-glucuronopyranosyl]-gymnemanol, 3-*O*-*β*-D-glucuronopyranosyl-22-*O*-tigloyl-gymnemanol and 3-*O*-*β*-D-glucuronopyranosyl-gymnemanol, respectively. The aglycone, gymnemanol, which is a new compound, was characterized as 3*β*,16*β*,22*α*,23,28-pentahydroxyolean-12-ene.

INTRODUCTION

Gymnema sylvestre R. Br., commonly known as 'Meshasringi' is distributed over most of India and it has a reputation in traditional medicine as a stomachic, a diuretic and as a remedy to control diabetes mellitus [1, 2]. The plant is popularly known as 'Gurmar' for its distinctive property of temporarily destroying the taste of sweetness. Studies have revealed that the water extract of the leaves of the plant inhibited absorption of glucose in the small intestine and suppressed the increase of blood sugar value after administration of sucrose in rats [3–5]. The plant extract has also been reported to have inhibitory action against glucan synthesis by glucosyltransferase from *Streptococcus mutans* [5] and has been suggested as a dentifrice to prevent dental caries [6]. An anti-eruodonic effect [5] of the extract and an antiviral effect [7] have also been suggested. The complex mixture of the active principles from a *Gymnema* extract, named gymnemic acid, was separated into four compounds, A_1–A_4, by Stöcklin *et al.* [8] who reported that these were D-glucuronides of a hexahydroxyolean-12-ene named gymnemegenin. Subsequently, a number of triterpenoid saponins have been isolated from the plant by different groups of workers [9–17]. They contain not only gymnemagnenin, but also 23-hydroxylongispinogenin, gymnestrogenin (pentahydroxyolean-12-ene) and a few dammarane derivatives as the aglycones. This paper reports the isolation and characterization of four new potential hypoglycaemic and antihyperglycaemic triterpenoid saponins from the plant. Preliminary results on the work have been presented [18].

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RESULTS AND DISCUSSION

The 50% hot aqueous ethanol extract of the air-dried leaves of the plant on successive column chromatography on Amberlite XAD-2, Sephadex LH-20 and silica gel, followed by preparative TLC and repeated HPLC, furnished four new tritepenoid saponins, designated gymnemasins A–D (**1**–**4**), besides the known gymnemic acids I–VI.

Gymnemasin A (**1**), $C_{47}H_{74}O_{17}$ showed by negative ion FAB-mass spectrometry [19, 20] a peak at *m/z* 909 attributable to $[M - H]^-$. The spectrum also showed some fragment ions which indicated that glucuronic acid was attached to the aglycone, and the glucose unit was linked to glucuronic acid. The positive ion FAB mass spectrum exhibited a discernible peak at *m/z* 933 ascribable to $[M + Na]^+$. Thus, the molecular weight of the compound was determined to be 910. The ^1H NMR spectrum displayed, besides an olefinic proton signal characteristic of H-12 of oleanenes, an olefinic proton at *δ*6.98 and two olefinic methyls at *δ*1.62 (3H, *d*, $J = 7$ Hz) and 1.88 (3H, *s*), indicating the presence of a tigloyl moiety. The ^{13}C NMR spectrum showed two singlets at *δ*167.9 and 173.3 assignable to tigloyl and glucuronyl carbonyls and two olefinic carbons at *δ*129.3(*s*) and 137.2(*d*) in addition to the C-12 and C-13 signals at *δ*123.7(*d*) and 143.8(*s*) (Table 1).

Mild alkaline hydrolysis of **1** yielded a prosapogenin identical with gymnemasin B (**2**) and tiglic acid. The prosapogenin **2** on mild acid hydrolysis afforded D-glucuronic acid and D-glucose as sugar constituents, identified by paper chromatography (PC) and GC, and a sapogenol, designated gymnemanol, mp 284–285°. The IR spectrum of gymnemanol showed the presence of hydroxyl functions, and the ^1HNMR spectrum displayed signals ascribed to six quaternary methyls.

The electron-impact mass spectrum showed the molecular ion at m/z 490 and retro-Diels–Alder fragment ions *a* and *b* at m/z 223 and 266, respectively, suggesting the presence of three hydroxyl groups in rings D/E and two hydroxyl groups in the part containing rings A/B. The ^{13}C NMR spectrum of the sapogenol revealed the presence of three secondary hydroxyls, two primary hydroxyls, six methyls, eight methylenes, three methines, and six sp^3 quaternary, one sp^2 methine and one sp^2 quaternary carbons. Comparison of the ^{13}C NMR data for gymnemanol with those for gymnestrogenin (3β,6β,21β,23,28-pentahydroxyolean-12-ene), [9] particularly for the hydroxyl bearing carbons, disclosed the difference in substitution in the ring E. This anomaly could satisfactorily be rationalized by placing a 22α (equatorial)-hydroxyl instead of 21β-group in gymnemanol and taking into consideration the hydroxyl substituent effects in ring E carbons of various hydroxyoleanenes [21]. Thus, the structure of gymnemanol was defined as 3β,16β,22α,23,28-pentahydroxyolean-12-ene (**5**).

Attachments of the glycosyl and tigloyl moieties in **1** were disclosed by comparison of the ^1H and ^{13}C NMR spectra of **1** and **5**. In the ^1H NMR spectrum an acylation shift [22] of + 0.88 ppm (from δ2.80 to 3.68) was observed for 22-H, and in the ^{13}C NMR spectrum a glycosylation shift [23, 24] of + 8.3 ppm (from δ73.7 to 82.0) was observed for C-3. Taking into consideration the FAB-mass spectral results, which indicated the sequence of the sugar units, and the ^{13}C NMR data for saponins **1** and **3**, which showed a glycosylation shift of + 9.0 ppm for C-3 of glucuronic acid, it could be inferred that C-1 of the glucose unit was linked to C-3 of glucuronic acid.

Permethylation of **1** by Hakomori's method [25] afforded the permethylate (**6**) which, on $LiAlH_4$ reduction, followed by acid hydrolysis, liberated 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-D-glucose identified by GC of the partially methylated alditol acetates and comparison of the R_t values [26, 27] with those of authentic samples.

The foregoing evidence led to the elucidation of the structure of gymnemasin A as 3-*O*-[β-D-glucopyranosyl (1 → 3)-β-D-glucuronopyranosyl]-22-*O*-tigloyl-gymnemanol (**1**).

Gymnemasin B (**2**), $C_{42}H_{68}O_{16}$, showed in its negative ion FAB mass spectrum the $[M - H]^-$ at m/z 827. Its ^1H and ^{13}C NMR spectra did not indicate the presence of a tigloyl moiety. On mild acid hydrolysis it furnished gymnemanol (**5**) as the aglycone, and D-glucuronic acid and D-glucose as the sugar constituents. The saponin was found to be identical with desacyl-gymnemasin A obtained by mild alkaline hydrolysis of gymnemasin A. Consequently, its structure was elucidated as 3-*O*-[β-D-glucopyranosyl(1 → 3)-β-D-glucuronopyranosyl]-gymnemanol (**2**). The ^{13}C NMR spectral data for the compound (Table 1) also were found to be compatible with the structure.

Gymnemasin C (**3**), $C_{41}H_{64}O_{12}$, displayed a $[M + Na]^+$ peak at m/z 771 in the positive ion FAB-mass spectrum, and the peak assigned to $[M - H]^-$ at m/z 747 in the negative ion spectrum. Usual alkaline and acid hydrolysis experiments and the ^1H and ^{13}C NMR data (Table 1) led to the elucidation of its structure as 3-*O*-β-D-glucuronopyranosyl-22-*O*-tigloyl-gymnemanol as shown.

Gymnemasin D (**4**), $C_{36}H_{58}O_{11}$, $[M - H]^-$ at m/z 665 in the negative FAB-mass spectrum was characterized as 3-*O*-β-D-glucuronopyranosyl-gymnemanol.

It is noteworthy that sapogenins so far isolated from *Gymnema* saponins are oleanane- and dammarane-type triterpenes. The other three oleanane sapogenins besides gymnemanol are gymnemagenin (3β,16β,21β,22α,23,28-hexahydroxyolean-12-ene), gymnestrogenin (3β,16β,-21β,23,28-pentahydroxy-olean-12-ene) and 23-hydroxy-longispinogenin (3β,16β,23,28-tetrahydroxy-olean-12-ene). To our knowledge, gymnemanol, which is isomeric with gymnestrogenin, is a new triterpene isolated for the first time from a natural source.

The total saponin fraction isolated from the leaves of *G. sylvestre* has been found to lower significantly the blood glucose level in normal, glucose fed hypoglycaemic and streptozotocin induced diabetic rats (unpublished data). Chattopadhyay *et al.* [28] recently reported hypoglycaemic and antihyperglycaemic effects of *C. sylvestre* leaf extract in rats.

EXPERIMENTAL

The plant material was collected from Madhya Pradesh, India, and identified at the Indian Botanic Garden, Howrah. A voucher specimen has been deposited at the herbarium of IICB.

All mps (uncorr.) were measured on a capillary mp apparatus. Optical rotations were determined on a JASCO DPI-360 digital polarimeter. IR spectra were recorded in KBr discs. ^1H NMR spectra were recorded on a JEOL FX-100 (99.6 MHz) instrument in $CDCl_3$ with TMS as int. standard. ^{13}C NMR spectra were recorded on a JEOL FX-100 Fourier-transform spectrometer operating at 25.05 MHz in pyridine-d_5 (C_5D_5N) (TMS as int. standard). FAB-MS were obtained at an accelerating voltage of 8 kV. A mixt. of glycerol and thioglycerol was used as a matrix and DMSO-d_6 as solvent. The samples were ionized by bombardment with Xe atoms produced by a saddle field ion source from Ion Tech. EI-MS were recorded at 70 eV. PC was performed on Whatman No. 1 paper with *n*-BuOH–pyridine–H_2O (6:4:3); a satd soln of aniline oxalate in H_2O was used for staining. GLC was performed on a Hewlett-Packard Model 5730A instrument using the column (i) ECNSS-M, 3% on Gas-Chrom Q at 190° for alditol acetates, and (ii) OV-225 on Gas-Chrom Q at 195° for partially methylated alditol acetates. HPLC was performed on a Spectra-Physics Model SP 8000B instrument with a column of Spherisorb S-10-ODS, a Micromeritics 771 refractive index detector and MeCN-H_2O (7:3) as mobile phase.

Isolation of the saponins. The air-dried powdered leaves of *G. sylvestre* (1.5 kg) were successively extracted

Table 1. ^{13}C NMR chemical shifts ($\delta_C \pm 0.1$) of gymnemasin A (**1**), gymnemasin B (**2**), gymnemasin C (**3**), gymnemasin D (**4**) and gymnemanol (**5**) measured in pyridine-d_5

C	1	2	3	4	5	C	1	2	3	4
1	39.0	39.2	39.0	39.1	38.5	GlcA-1	105.2	105.3	105.4	105.5
2	26.2	26.1	26.2	26.2	27.5	GlcA-2	74.3	74.3	75.4	75.5
3	82.0	82.1	82.1	82.2	73.7	GlcA-3	87.2	87.3	78.2	78.3
4	43.5	43.5	43.4	43.5	42.8	GlcA-4	71.8*	71.8*	73.2	73.2
5	47.9	47.8	47.8	47.7	48.8	GlcA-5	77.5	77.5	77.8	77.9
6	18.5	18.6	18.7	18.7	18.7	GlcA-6	173.2	173.3	173.2	173.3
7	32.7	32.7	32.8	32.6	32.8	Glc-1	105.5	105.5		
8	40.2	40.1	40.3	40.2	40.0	Glc-2	75.5	75.4		
9	47.2	47.4	47.5	47.6	47.3	Glc-3	78.4†	78.4†		
10	37.2	37.2	37.3	37.1	37.1	Glc-4	71.4*	71.4*		
11	24.2	24.1	24.1	24.1	23.8	Glc-5	78.2†	78.3†		
12	123.7	123.6	123.8	123.7	123.6	Glc-6	62.1	62.2		
13	143.0	143.2	143.4	143.4	143.3	1′	167.9		167.8	
14	42.5	42.6	42.7	43.8	42.7	2′	129.3		129.2	
15	36.2	36.1	36.3	36.2	36.1	3′	137.2		137.3	
16	67.6	67.6	67.7	67.8	67.7	4′	12.4		12.4	
17	47.2	46.7	47.3	46.8	46.5	5′	14.2		14.1	
18	42.6	43.4	42.5	43.4	43.5					
19	46.8	46.7	46.9	46.8	46.6					
20	31.2	31.4	31.3	31.5	31.4					
21	41.8	43.5	41.8	43.4	43.6					
22	71.4	70.5	71.5	70.5	70.3					
23	64.3	64.4	64.4	64.4	68.2					
24	13.6	13.5	13.7	13.6	13.4					
25	16.3	16.3	16.4	16.4	16.2					
26	17.2	17.1	17.2	17.1	17.0					
27	27.2	27.2	27.3	27.2	27.1					
28	60.2	59.3	60.3	59.4	59.7					
29	33.4	33.3	33.4	33.5	33.5					
30	24.8	24.7	24.9	24.8	24.9					

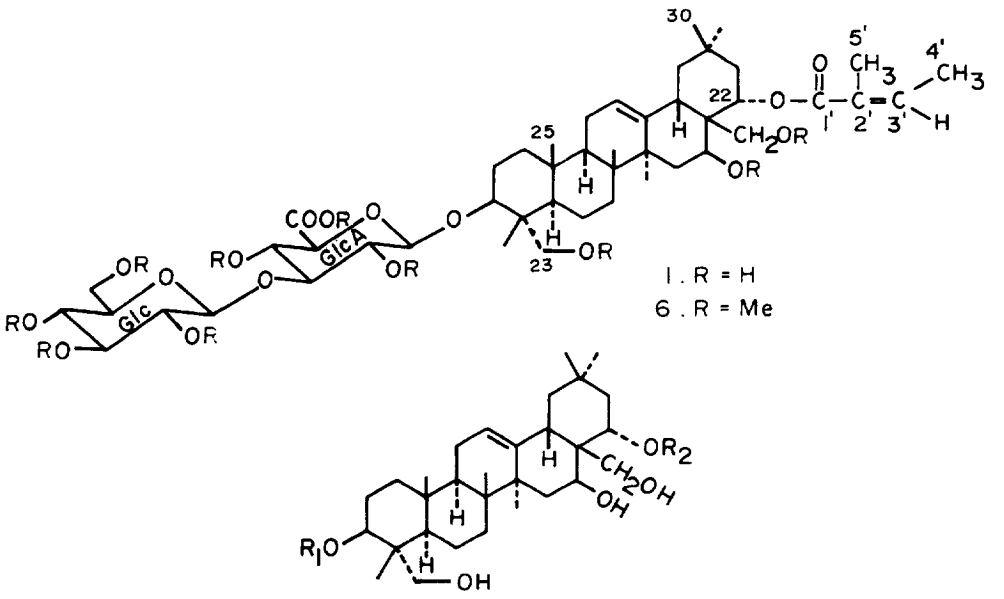
GlcA = glucuronic acid; Glc = glucose.
*†May be interchanged within the same column.

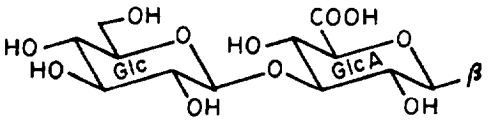
with petrol, $CHCl_3$ and EtOH–H_2O (1:1). The EtOH extract on removal of the solvent under red. pres. was partitioned between *n*-BuOH and H_2O. The organic layer was concd to dryness. The residue was dissolved in MeOH and passed through an Amberlite XAD-2 column and eluted with MeOH. The crude saponins thus obtained were passed through a column of Sephadex LH-20 and eluted with MeOH. The residue was subjected to prep. TLC on silica gel followed by repeated HPLC on the S-10-ODS column using 25–30% aq. MeCN as mobile phase. The new saponins, **1** (125 mg), **2** (85 mg), **3** (110 mg) and **4** (72 mg), besides the known gymnemic acids I–VI [9, 10], were thus obtained.

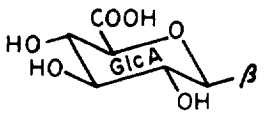
Gymnemasin A (**1**). An amorphous powder, mp 215–217°, $[\alpha]_D + 15°$ (*c* 1.5 in MeOH). IR ν_{max} cm^{-1}: 3400 (OH), 1715 (C=O), 1600 (C=C). ^1H NMR: δ(pyridine-d_5) 0.88 (3H, *s*), 0.92 (3H, *s*), 0.94 (3H, *s*), 1.04 (3H, *s*), 1.14 (3H, *s*), 1.31 (3H, *s*), 1.62 (3H, *d*, $J = 7$ Hz), 1.88 (3H, *s*), 7.02 (1H, *q*, $J = 7$ Hz); ^{13}C NMR: see Table 1. Negative FAB-MS *m/z* 909 $[M - H]^-$; positive FAB-MS *m/z* 933 $[M + Na]^+$ (Found: C, 60.81; H, 8.21; $C_{47}H_{74}O_{17}\cdot H_2O$ requires C, 60.76; H, 8.26%).

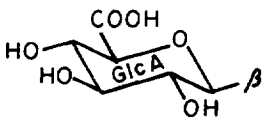
Alkaline hydrolysis of gymnemasin A (**1**). Compound (**1**) (150 mg) was hydrolysed with 6% KOH in aq. EtOH (20 ml) under reflux for 3 hr. The reaction mixt. was acidified with 5% HCl and extracted first with $C_2H_4Cl_2$ and then with *n*-BuOH. The solvents of both extracts were removed under red. pres. and the residues were purified by CC on silica gel. The $C_2H_4Cl_2$ extract yielded tiglic acid (mp, co-TLC) and then *n*-BuOH extract afforded a prosapogenin identical with gymnemasin B (**2**).

Gymnemanol (**5**). The prosapogenin **2** (120 mg), obtained as described above, was hydrolysed with 5% HCl in aq. EtOH at water-bath temp. for 3 hr. The usual work-up followed by CC purification on silica gel afforded **5** which crystallized from MeOH as microneedles, mp 284–285°, $[\alpha]_D + 51.5°$ (*c* 1.0 in MeOH). IR ν_{max} cm^{-1}: 3350 (OH). ^1H NMR (pyridine-d_5): δ 5.35 (1H, *t*-like, H-12), 4.80 (1H, *dd*, $J = 11$, 5 Hz, H-16), 3.80–4.78 (6H, *m*), 0.90 (3H, *s*), 0.92 (3H, *s*), 0.96 (3H, *s*), 1.02 (3H, *s*), 1.12 (3H, *s*), 1.30 (3H, *s*); EIMS *m/z* (rel. int.): 490 $[M]^+$ (10), 472 $[M - H_2O]^+$ (6), 454 $[M - 2H_2O]^+$ (18), 441 $[M - H_2O - CH_2OH]^+$ (25), 436 $[M - 3H_2O]^+$ (12), 266 $[b]^+$ (35), 223 $[a]^+$ (8), 207 $[b - H_2O - CH_2OH]^+$(100), 205 $[a - H_2O]^+$(30); ^{13}C NMR: see Table 1. (Found: C, 73.49; H, 10.31; $C_{30}H_{50}O_5$ requires C, 73.43; H, 10.27%).

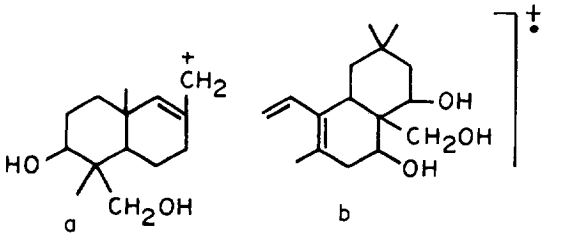


2. R_1 =  , R_2 = H

3. R_1 =  , R_2 = Tigloyl

4. R_1 =  , R_2 = H

5. R_1 = R_2 = H



The filtrate from the acid hydrolysate was neutralized with Ag_2CO_3, filtered and a portion of the filtrate concd under red. pres. and tested for carbohydrates by PC with *n*-BuOH–pyridine–H_2O (6:4:3). Spots corresponding to D-glucuronic acid and D-glucose were detected by comparison with those of authentic samples. The other portion of the conc. filtrate was reduced with $NaBH_4$ and worked-up as usual. The residue was acetylated with Ac_2O–pyridine (1:1) at water-bath temp. for 1 hr, dried *in vacuo*, purified by chromatography over silica gel and subjected to GC analysis using column (i). Only one peak corresponding to glucitol acetate was detected. Under

the conditions employed no product from the glucuronic acid portion would be expected to elute.

Permethylation of gymnemasin A (**1**). Compound **1** (150 mg) was permethylated following the method of ref. [25] using MeI–DMSO–NaH. Usual work-up followed by purification by silica gel CC and elution with petrol–EtOAc (3:2) furnished the permethylate (**6**) as a powder (122 mg), mp 155–157° (no hydroxyl absorption in the IR spectrum). ^{1}H NMR ($CDCl_3$): δ3.15, 3.20, 3.24, 3.30, 3.34, 3.38, 3.40, 3.45 (all *s*, 10 × OMe), 4.40, 4.44 (1H each, *d*, J = 7 Hz) (H-1 of glucuronide, and H-1 of glucoside).

Reduction of the permethylate **6** *with* $LiAlH_4$ *and hydrolysis of the product.* Compound **6** (110 mg) in dry Et_2O (15 ml) was treated with a suspension of $LiAlH_4$ (110 mg) in dry Et_2O and the reaction mixt. was stirred at room temp. (27°C) for 1 hr. A few drops of aq. Et_2O were then added and acidified with aq. 20% H_2SO_4. Extraction with Et_2O followed by usual work-up yielded the reduction product as a semi-solid, which was hydrolysed with 2 M HCl in aq. MeOH (20 ml) under reflux. The reaction mixt. was cooled, evapd to dryness under red. pres., diluted with H_2O and filtered. The filtrate was concd and the product reduced with $NaBH_4$. Usual work-up followed by acetylation with Ac_2O–pyridine yielded a mixt. of alditol acetates, which was subjected to GC analysis using column (ii). Two peaks were detected, corresponding to the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-D-glucose by comparison of their R_t values with those of authentic samples.

Gymnemasin B (**2**). Amorphous powder, mp 221–222°, $[\alpha]_D$ + 18.5° (*c* 1.2 in MeOH). IR ν_{max} cm^{-1}: 3420 (OH), 1710 (C=O). ^{1}H NMR (pyridine-d_5): δ 0.86 (3H, *s*), 0.90 (3H, *s*), 0.94 (3H, *s*), 1.04 (3H, *s*), 1.12 (3H, *s*), 1.30 (3H, *s*); ^{13}C NMR: see Table 1; negative FAB-MS *m/z* 827 $[M - H]^-$ (found: C, 60.89; H, 8.24; $C_{42}H_{68}O_{16}$ requires C, 60.85; H, 8.27%).

Gymnemasin C (**3**). Amorphous powder, mp 212–214°, $[\alpha]_D$ + 12.5° (*c* 1.0 in MeOH); IR ν_{max} cm^{-1}: 3410 (OH); 1715 (C=O). ^{1}H NMR: (pyridine-d_5): δ0.88 (3H, *s*), 0.90 (3H, *s*), 0.93 (3H, *s*), 1.02 (3H, *s*), 1.28 (3H, *s*), 1.30 (3H, *s*), 1.64 (3H, *s*), 1.86 (3H, *s*), 7.01 (1H, *q*, J = 7 Hz); ^{13}C NMR: see Table 1; positive FAB-MS *m/z* 771 $[M + Na]^+$, negative FAB-MS *m/z* 747 $[M - H]^-$ (Found: C, 65.71, H, 8.64; $C_{41}H_{64}O_{12}$ requires C, 65.74; H, 8.61%).

Gymnemasin D (**4**). Amorphous powder, mp 220–221°, $[\alpha]_D$ + 8° (*c* 0.9 in MeOH). IR ν_{max} cm^{-1}: 3425 (OH), 1715 (C=O). ^{1}H NMR (pyridine-d_5): δ0.86 (3H, *s*), 0.89 (3H, *s*), 0.93 (3H, *s*), 1.04 (3H, *s*), 1.14 (3H, *s*), 1.30 (3H, *s*); ^{13}C NMR: see Table 1; negative FAB-MS *m/z* 665 $[M - H]^-$ (Found: C, 64.79; H, 8.80; $C_{36}H_{58}O_{11}$ requires C, 64.88; H, 8.77%).

Alkaline and acid hydrolysis of gymnemasins B–D (**2–4**). Alkaline and acid hydrolysis of **2–4** (50 mg each) were carried out as described for **1**. The aglycone obtained in each case was characterized as gymnenanol (**5**); tiglic acid was identified as the acyl moiety for **3**, D-glucuronic acid, D-glucose as sugar constituents for **2**, and D-glucose as sugar moiety for each of **3** and **4**.

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