Medicinal Foodstuffs. IV.[1] Fenugreek Seed. (1): Structures of Trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, New Furostanol Saponins from the Seeds of Indian *Trigonella foenum-graecum* L.

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Six new furostanol saponins called trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb were isolated from a medicinal foodstuff, fenugreek seed, the seed of *Trigonella foenum-graecum* L. (Leguminosae) originating from India, together with two known saponins, glycoside D and trigofoenoside A. The structures of trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb were determined on the basis of chemical and physicochemical evidence as 26-*O*-*β*-D-glucopyranosyl-(25*S*)-5*α*-furostane-2*α*,3*β*,22*ξ*,26-tetraol 3-*O*-[*β*-D-xylopyranosyl (1→6)]-*β*-D-glucopyranoside, 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*α*-furostane-2*α*,3*β*,22*ξ*,26-tetraol 3-*O*-[*β*-D-xylopyranosyl (1→6)]-*β*-D-glucopyranoside, 26-*O*-*β*-D-glucopyranosyl-(25*S*)-5*β*-furostane-3*β*,22*ξ*,26-triol 3-*O*-[*β*-D-xylopyranosyl (1→6)]-*β*-D-glucopyranoside, 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*β*-furostane-3*β*,22*ξ*,26-triol 3-*O*-[*β*-D-xylopyranosyl (1→6)]-*β*-D-glucopyranoside, 26-*O*-*β*-D-glucopyranosyl-(25*S*)-5*α*-furostane-3*β*,22*ξ*,26-triol 3-*O*-[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside, and 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*α*-furostane-3*β*,22*ξ*,26-triol 3-*O*-[*α*-L-rhamnopyranosyl (1→2)]-*β*-D-glucopyranoside, respectively.

Key words *Trigonella foenum-graecum*; fenugreek; trigoneoside; medicinal foodstuff; furostanol saponin; Leguminosae

Trigonella foenum-graecum L. (fenugreek, Leguminosae), which is an annual herbaceous plant 30 to 60 cm tall, has been widely cultivated in Asia, Africa, and Mediterranean countries. The seeds of this plant (Japanese and Chinese name "胡蘆巴") have been used as a spice from ancient times. Recently, the food industry has made extensive use of fenugreek as a seasoning in coffee extract, vanilla extract, and chutney and as a flavouring in tobacco, artificial maple syrup, and curry. In Chinese traditional medicine, fenugreek seeds have been prescribed for tonic and stomachic purposes, while this plant has been used for a long time as an antipyretic, laxative, and strengthening agent in the Mediterranean area. The chemical constituents of fenugreek from various countries have been pursued extensively[2] and many steroidal saponins, which are the principal constituents of this plant, have been isolated.[3]

As a part of our continuing studies on the bioactive constituents of medicinal foodstuffs,[1,4] we have isolated six new furostanol saponins called trigoneosides Ia (**1**), Ib (**2**), IIa (**3**), IIb (**4**), IIIa (**5**), and IIIb (**6**) from Indian fenugreek seeds. This paper describes the structure elucidation of these trigoneosides (**1**—**6**) on the basis of chemical and physicochemical evidence.[5]

The methanolic extract of fenugreek seeds cultivated in India was subjected to Diaion HP-20 column chromatography to give the glycosidic fraction (6.4%), which was further separated by ordinary silica-gel column chromatography to furnish seven fractions (fr. 1—7). Fraction 2 (0.72%) was purified by reversed-phase silica-gel column chromatography followed by HPLC to give trigoneosides Ia (**1**, 0.022%), Ib (**2**, 0.055%), IIa (**3**, 0.082%), IIb (**4**, 0.024%), IIIa (**5**, 0.015%), and IIIb (**6**, 0.009%), together with glycoside D[6] (**7**, 0.042%) and trigofoenoside A[3e] (**8**, 0.084%).

Trigoneosides Ia (1) and Ib (2) Trigoneoside Ia (**1**) was obtained as a white powder, and, based on TLC examination using the Ehrlich reagent,[7] was deduced to possess a furostanol structure. The IR spectrum of **1** showed strong absorption bands at 3405, 1078, and 1040 cm^{-1} suggestive of oligoglycosidic structure. In the negative-mode and positive-mode FAB-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 905 $(M-H)^-$ and *m/z* 929 $(M+Na)^+$, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{44}H_{74}O_{19}$. Furthermore, fragment ion peaks at *m/z* 773 $(M-C_5H_9O_4)^-$ and *m/z* 611 $(M-C_{11}H_{19}O_9)^-$, which were derived by cleavage of the glycosidic linkage at the 6′ and

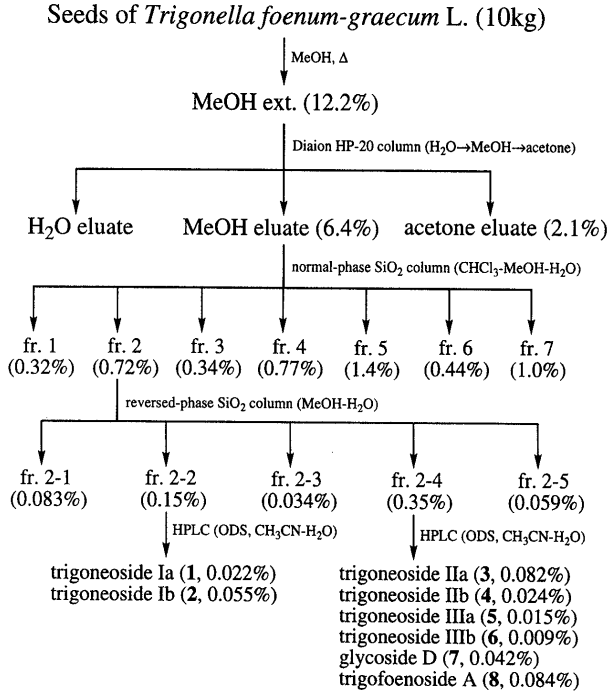

Chart 1

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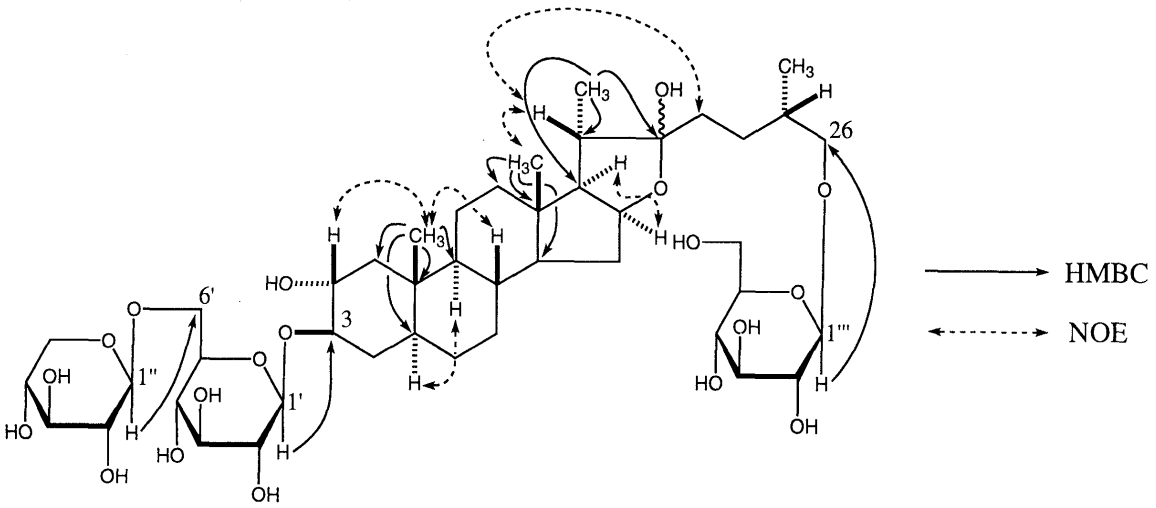


Fig. 1. Results of HMBC and ROESY Experiments on **1**

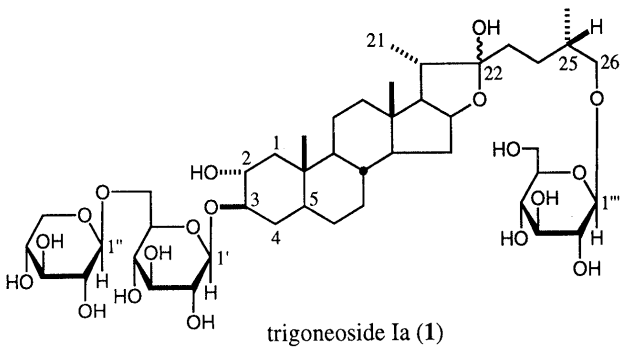
3-positions, were observed in the negative-mode FAB-MS of **1**. The methanolysis of **1** with 9% hydrogen chloride in dry methanol gave methyl glucoside and methyl xyloside in a *ca.* 2:1 ratio,[8] while acid hydrolysis of **1** with 2 N hydrochloric acid–dioxane (1:1) liberated neogitogenin (**9**).[9]

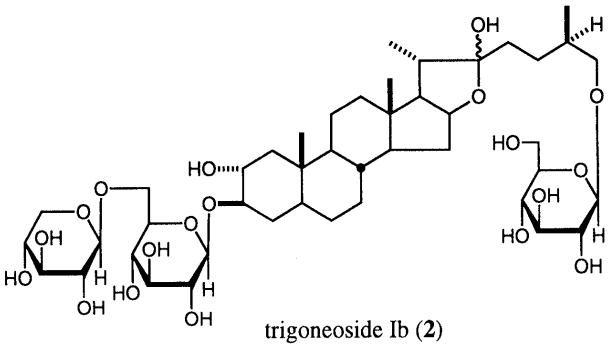
The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR spectra (Table 1) of **1**, which were assigned by various NMR analytical methods,[10] showed signals due to a furostane-2,3,22,26-tetraol part [δ 1.16 (m), 2.15 (dd, J=4.0, 10.6 Hz) (1-H_2), 3.95 (m, 2-H), 3.70 (m, 3-H), 1.42 (m), 1.76 (dd, J=5.2, 11.6 Hz) (4-H_2), 1.06 (m, 5-H), 0.86 (s, 18-H_3), 0.72 (s, 19-H_3), 1.30 (d, J=7.0 Hz, 21-H_3), 1.93 (m, 25-H), 3.48 (dd, J=7.0, 9.5 Hz), 4.07 (m) (26-H_2), 1.03 (d, J=6.4 Hz, 27-H_3)], 3-*O*-β-D-glucopyranosyl moiety [δ 4.95 (d, J=7.6 Hz, 1′-H), 3.97, 4.98 (both m, 6′-H_2)], 6′-*O*-β-D-xylopyranosyl moiety [δ 4.80 (d, J=7.3 Hz, 1″-H)], and 26-*O*-β-D-glucopyranosyl moiety [δ 4.81 (d, J=7.9 Hz, 1‴-H), 4.38 (dd, J=5.4, 11.8 Hz), 4.54 (dd, J=2.6, 11.8 Hz) (6‴-H_2)]. The 3,26-bisdesmoside structure of **1** was characterized by a heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-range correlations were observed between the 1″-proton and the 6′-carbon, between the 1′-proton and the 3-carbon, and between the 1‴-proton and the 26-carbon; the assignments of the anomeric protons were confirmed by ^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA.[10] Furthermore, the furostanol structure of **9** was characterized by HMBC and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments as shown in Fig. 1. Finally, by comparison of the NMR data for **1** with those for related furostanol saponins,[3,6,11] the structure of trigoneoside Ia was elucidated as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-2α,3β,22ξ,26-tetraol 3-*O*-[β-D-xylopyranosyl (1→6)]-β-D-glucopyranoside (**1**).

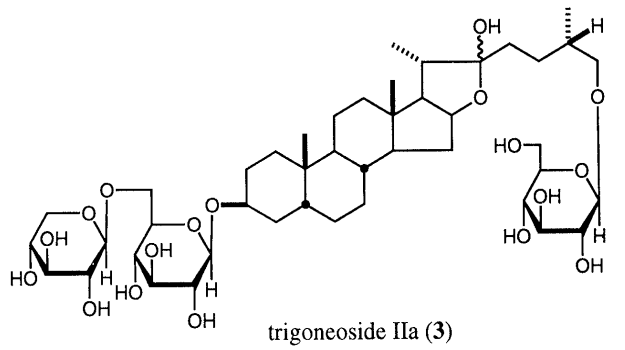
Trigoneoside Ib (**2**), obtained as a white powder, was deduced to possess a furostanol structure based on the positive Ehrlich test. The IR spectrum of **2** was found to be similar to that of **1**. The negative-mode and positive-mode FAB-MS of **2** showed quasimolecular ion peaks at m/z 905 $(M-H)^-$ and m/z 929 $(M+Na)^+$, respectively, and fragment ion peaks at m/z 773 $(M-C_5H_9O_4)^-$ and m/z 611 $(M-C_{11}H_{19}O_9)^-$ were observed in the negative-mode FAB-MS. High-resolution MS analysis revealed the molecular formula of **2** to be $C_{44}H_{74}O_{19}$, which was the same as that of **1**. The carbon and proton signals of **2** in the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[10] were shown to be superimposable on those of **1**, except for the 26-protons [δ 3.63 (dd, J=6.1, 9.2 Hz), 3.95 (m) (26-H_2)]. Furthermore, long-range correlations were observed between the following protons and carbons (1″-H and 6′-C, 1′-H and 3-C, 1‴-H and 26-C) in the HMBC experiment on **2**. Methanolysis of **2** liberated methyl glucoside and methyl xyloside in a *ca.* 2:1 ratio,[8] while gitogenin (**10**),[12] which is the 25*R*-stereoisomer of **9**, was obtained by acid hydrolysis of **2**. Consequently, the structure of trigoneoside Ib was elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-2α,3β,22ξ,26-tetraol 3-*O*-[β-D-xylopyranosyl (1→6)]-β-D-glucopyranoside (**2**).

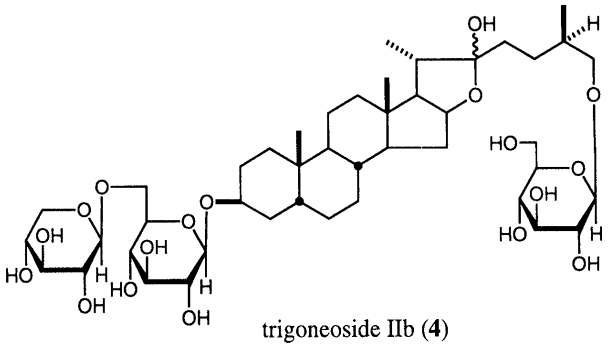
Trigoneosides IIa (3) and IIb (4) Trigoneosides IIa (**3**) and IIb (**4**), which were each isolated as a white powder, gave a positive Ehrlich test. The IR spectra of **3** and **4** showed absorption bands due to hydroxyl groups. Trigoneosides IIa (**3**) and IIb (**4**) were found to have the same molecular formula $C_{44}H_{74}O_{18}$, which was determined from their positive-mode and negative-mode FAB-MS and by high-resolution MS measurement. Thus, in the positive-mode FAB-MS of **3** and **4**, the quasimolecular ion peak was observed at m/z 913 $(M+Na)^+$, while their negative-mode FAB-MS showed the quasimolecular ion peak at m/z 889 $(M-H)^-$ in addition to fragment ion peaks at m/z 757 $(M-C_5H_9O_4)^-$, m/z 727 $(M-C_6H_{11}O_5)^-$, and m/z 595 $(M-C_{11}H_{19}O_9)^-$. Methanolysis of **3** and **4** liberated methyl glucoside and methyl xyloside in a *ca.* 2:1 ratio.[8] Acid hydrolysis of **3** gave sarsasapogenin (**11**),[13] while smilagenin (**12**),[14] which is the 25*R*-isomer of **11**, was obtained by the acid hydrolysis of **4**.

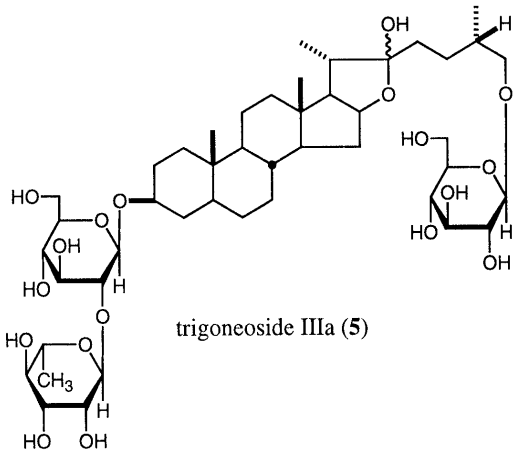
The proton and carbon signals due to the sugar moieties in the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1)[10] spectra of **3** and **4** were similar to those of **1** and **2**, and HMBC experiments showed long-range correlations between the following protons and carbons (1″-H [δ 5.04 (d)] and 6′-C; 1′-H [δ 4.87 (d)] and 3-C; 1‴-H [δ 4.80


trigoneoside Ia (**1**)


trigoneoside Ib (**2**)


trigoneoside IIa (**3**)


trigoneoside IIb (**4**)


trigoneoside IIIa (**5**)

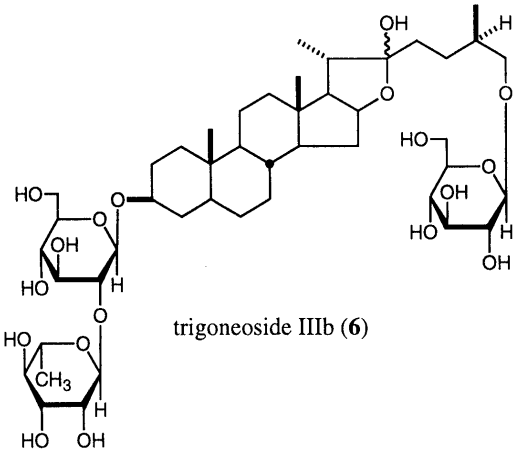
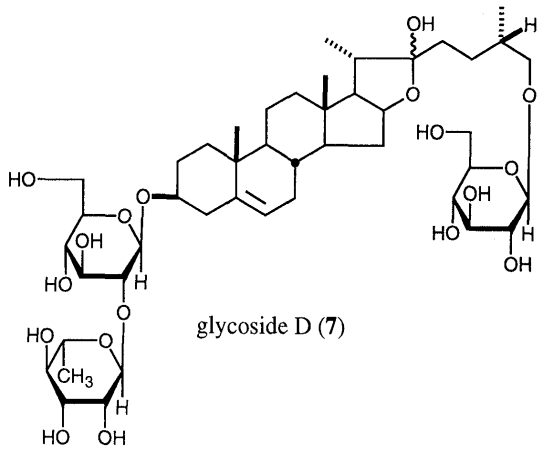

trigoneoside IIIb (**6**)

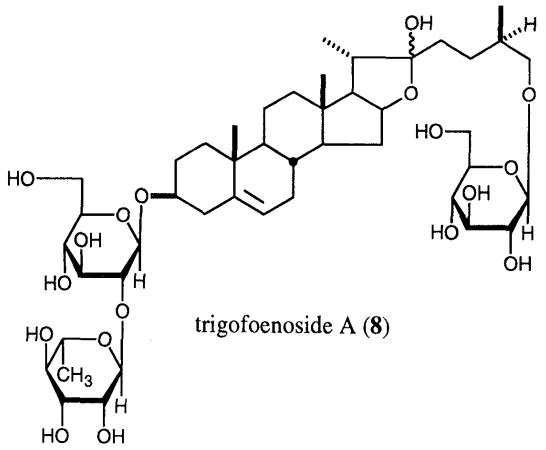
Chart 1

(d)] and 26-C). The proton signals assignable to the 26-methylene group [δ 3.48 (dd, $J=7.1$, 9.5 Hz), 4.08 (dd, $J=5.6$, 9.5 Hz)] in the ^{1}H-NMR spectrum of **3** were very similar to those of **1**, while the 26-methylene signals [δ 3.62 (dd, $J=6.1$, 9.5 Hz), 3.94 (dd, $J=7.0$, 9.5 Hz)] of **4** were very similar to those of **2**. On the basis of the above evidence, the structures of trigoneosides IIa and IIb were formulated as 26-β-D-glucopyranosyl-(25*S*)-5β-furostane-3β,22ξ,26-triol 3-*O*-[β-D-xylopyranosyl (1→6)]-β-D-glucopyranoside (**3**) and its 25*R*-isomer (**4**).

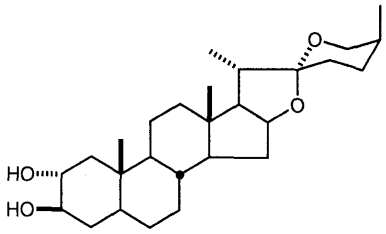
Trigoneosides IIIa (5) and IIIb (6) Trigoneosides IIIa (**5**) and IIIb (**6**), each obtained as a white powder, were positive in the Ehrlich test. They were found to have the same molecular formula, $C_{45}H_{76}O_{18}$, which was determined from the quasimolecular ion peaks in their negative-mode FAB-MS [*m/z* 903 $(M-H)^-$] and positive-mode FAB-MS [*m/z* 927 $(M+Na)^+$] and by high-resolution MS measurement. Furthermore, the negative-mode FAB-MS of **5** and **6** showed fragment ion peaks at *m/z* 757 $(M-C_6H_{11}O_4)^-$, 741 $(M-C_6H_{11}O_5)^-$, and 595 $(M-C_{12}H_{21}O_9)^-$. Methanolysis of **5** and **6** liberated methyl glucoside and methyl rhamnoside in a *ca.* 2:1 ratio.[8] Acid hydrolysis of **5** furnished neotigogenin (**13**)[9] having 25*S*-configuration, while acid hydrolysis of **6** gave tigogenin (**14**)[15] having 25*R*-configuration. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra on **5** were shown to be superimposable on those of **6**, except for the signals of the 26-methylene protons in the ^{1}H-NMR. Namely, the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[10] of **5** and **6** showed signals



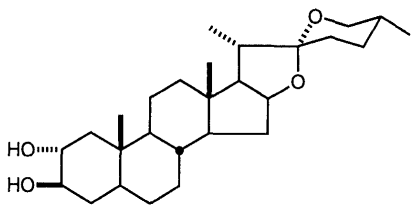
glycoside D (**7**)



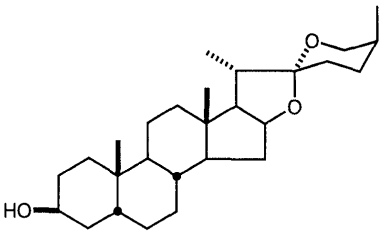
trigofoenoside A (**8**)



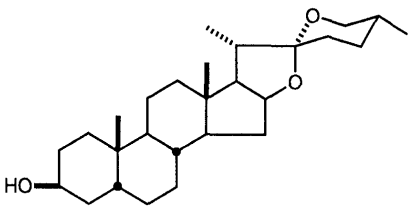
neogitogenin (**9**)



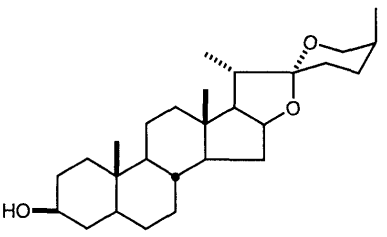
gitogenin (**10**)



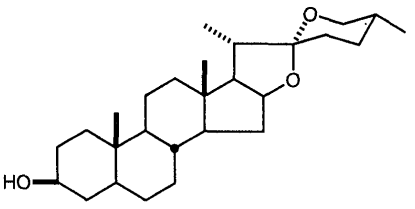
sarsasapogenin (**11**)



smilagenin (**12**)



neotigogenin (**13**)



tigogenin (**14**)

Chart 2

assignable to a 3-*O*-*β*-D-glucopyranosyl moiety [**5**: *δ* 5.07 (d, *J*=7.4 Hz); **6**: *δ* 5.08 (d, *J*=7.3 Hz) (1′-H)], 2′-*O*-*α*-L-rhamnopyranosyl moiety [**5**: *δ* 6.36 (br s); **6**: *δ* 6.37 (br s) (1″-H)], and 26-*O*-*β*-D-glucopyranosyl moiety [**5**: *δ* 4.82 (d, *J*=7.7 Hz); **6**: *δ* 4.83 (d, *J*=7.6 Hz) (1‴-H)]. The HMBC experiment on **5** and **6** showed long-range correlations between the 1″-proton and the 2′-carbon, between the 1′-proton and the 3-carbon, and between the 1‴-proton and the 26-carbon. Comparison of the NMR data for **5** and **6** with those for **1**, **2**, **3**, **4**, and related furostanol saponins[2,6,11] led us to formulate the structures of trigoneosides IIIa and IIIb as 26-*O*-*β*-D-glucopyranosyl-(25*S*)-5*α*-furostane-3*β*,22*ξ*,26-triol 3-*O*-[*α*-L-rhamnopyranosyl (1→2)]-*β*-D-glucopyranoside (**5**) and its 25*R*-isomer (**6**).

In parallel studies, we have chemically investigated the saponin constituents isolated from the highly polar fractions (Chart 1) and we also intend to examine the

Table 1. ^{13}C-NMR Data for Trigoneosides Ia (**1**), Ib (**2**), IIa (**3**), IIb (**4**), IIIa (**5**), and IIIb (**6**)

	1	2	3	4	5	6		1	2	3	4	5	6
C-1	45.3	45.3	31.0	31.0	37.3	37.3	3-*O*-Glc-1″	104.8	104.8	103.1	103.1	99.9	99.9
C-2	71.1	71.1	27.0	27.0	30.0	30.0	2′	74.9	74.8	75.2	75.2	78.1	78.1
C-3	87.9	87.9	74.6	74.6	77.0	77.0	3′	78.6	78.6	78.6	78.6	78.3	78.4
C-4	34.6	34.6	30.6	30.6	34.5	34.5	4′	72.2	72.2	71.8	71.7	72.0	72.0
C-5	44.6	44.7	37.0	37.0	44.6	44.6	5′	76.4	76.5	77.2	77.2	79.7	79.7
C-6	28.0	28.0	27.0	27.0	29.0	29.0	6′	70.5	70.4	69.9	69.9	62.9	62.9
C-7	32.2	32.2	26.8	26.8	32.5	32.5	6′-*O*-Xyl-1″	105.4	105.4	105.8	105.7		
C-8	34.5	34.5	35.5	35.5	35.3	35.3	2″	75.3	75.3	74.9	74.9		
C-9	54.3	54.3	40.3	40.3	54.5	54.5	3″	77.9	77.9	78.2	78.2		
C-10	36.8	36.8	35.2	35.2	36.0	36.0	4″	71.1	71.1	71.1	71.1		
C-11	21.4	21.4	21.1	21.1	21.3	21.3	5″	67.2	67.1	67.1	67.1		
C-12	40.1	40.1	40.4	40.4	40.3	40.3	2′-*O*-Rha-1″					102.2	102.2
C-13	41.1	41.1	41.2	41.2	41.1	41.1	2″					72.6	72.6
C-14	56.2	56.2	56.4	56.4	56.4	56.4	3″					72.8	72.9
C-15	32.4	32.4	32.4	32.4	32.4	32.4	4″					74.2	74.2
C-16	81.1	81.1	81.2	81.2	81.2	81.1	5″					69.5	69.5
C-17	63.9	64.0	64.0	64.0	64.0	64.0	6″					18.7	18.7
C-18	16.7	16.7	16.7	16.7	16.7	16.7	26-*O*-Glc-1‴	105.1	104.9	105.1	104.9	105.1	105.0
C-19	13.3	13.3	23.9	23.9	12.5	12.5	2‴	75.2	75.2	75.2	75.2	75.2	75.2
C-20	40.7	40.7	40.7	40.7	40.7	40.7	3‴	78.4	78.4	78.6	78.6	78.6	78.6
C-21	16.5	16.4	16.5	16.5	16.5	16.5	4‴	71.7	71.7	71.7	71.7	71.7	71.8
C-22	110.6	110.6	110.7	110.7	110.6	110.6	5‴	78.4	78.4	78.4	78.4	78.5	78.5
C-23	37.1	37.2	37.1	37.2	37.2	37.2	6‴	62.8	62.8	62.8	62.8	62.8	62.8
C-24	28.3	28.4	28.3	28.4	28.3	28.4							
C-25	34.4	34.3	34.4	34.3	34.3	34.3							
C-26	75.4	75.3	75.4	75.3	75.4	75.3							
C-27	17.5	17.5	17.5	17.5	17.5	17.5							

biological activity of these new compounds. The results will be reported in a forthcoming paper.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1)]

Isolation of Trigoneosides Ia (1), Ib (2), IIa (3), IIb (4), IIIa (5), and IIIb (6) and Known Compounds (7, 8) from the Seeds of *Trigonella foenum-graecum* L. The seeds of *Trigonella foenum-graecum* L. (10 kg, cultivated in India and purchased from Honso Pharmaceutical Co., Ltd., Nagoya) were crushed and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (1.22 kg, 12.2%), and a part of it (342.7 g) was subjected to Diaion HP-20 column chromatography [3 kg (Nippon Rensou Co.), $H_2O \rightarrow$MeOH$\rightarrow$acetone] to give the H_2O eluate, MeOH eluate (179.3 g, 6.4%), and acetone eluate (59.1 g, 2.1%). Normal-phase silica-gel column chromatography {BW-200 (Fuji Silysia Ltd., 3 kg), $CHCl_3$–MeOH–H_2O [7 : 3 : 1 (lower layer)$\rightarrow$65 : 35 : 10 (lower layer)]$\rightarrow$MeOH} of the MeOH eluate (169 g) give seven fractions [fr. 1 (8.4 g, 0.32%), fr. 2 (19.1 g, 0.72%), fr. 3 (8.9 g, 0.34%), fr. 4 (20.4 g, 0.77%), fr. 5 (36.2 g, 1.4%), fr. 6 (11.5 g, 0.44%), fr. 7 (26.7 g, 1.0%)]. Fraction 2 (19.1 g) was separated by reversed-phase silica-gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 500 g), MeOH–H_2O (50 : 50$\rightarrow$65 : 35$\rightarrow$80 : 20, v/v)$\rightarrow$MeOH] to give five fractions: fr. 2-1 (2.2 g, 0.083%), fr. 2—2 (3.9 g, 0.15%), fr. 2-3 (0.9 g, 0.034%), fr. 2-4 (9.1 g, 0.35%), and fr. 2-5 (1.6 g, 0.059%). Fraction 2-2 was further purified by HPLC [YMC-Pack ODS-A (250 × 25 mm i.d., YMC Co., Ltd.), CH_3CN–H_2O (25 : 75, v/v] to give trigoneosides Ia (**1**, 567.8 mg, 0.022%) and Ib (**2**, 1444 mg, 0.055%). Repeated HPLC [1) YMC-Pack ODS-A (250 × 25 mm i.d.), CH_3CN–H_2O (30 : 70, v/v); 2) YMC-Pack ODS-AL (250 × 25 mm i.d.), CH_3CN–H_2O (25 : 75, v/v)] separation of fr. 2-4 afforded trigoneosides IIa (**3**, 2145 mg, 0.082%), IIb (**4**, 635.3 mg, 0.024%), IIIa (**5**, 387.9 mg, 0.015%), and IIIb (**6**, 236.2 mg, 0.009%), glycoside D[6)] (**7**, 0.042%), and trigofoenoside A[3e)] (**8**, 0.084%). The known compounds (**7**, **8**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[3e,6)]

Trigoneoside Ia (**1**): A white powder, $[\alpha]_D^{26}$ −41.8° (c = 0.34, pyridine). High-resolution positive-mode FAB-MS: Calcd for $C_{44}H_{74}NaO_{19}$ $(M+Na)^+$: 929.4722; Found: 929.4742. IR (KBr) cm^{-1}: 3405, 2930, 1078, 1040. ^{1}H-NMR (pyridine-d_5, δ): 0.72, 0.86 (3H each, both s, 19, 18-H_3), 1.03 (3H, d, J = 6.4 Hz, 27-H_3), 1.06 (1H, m, 5-H), 1.16 (1H, m), 2.15 (1H, dd, J = 4.0, 10.6 Hz) (1-H_2), 1.30 (3H, d, J = 7.0 Hz, 21-H_3), 1.42 (1H, m), 1.76 (1H, dd, J = 5.2, 11.6 Hz) (4-H_2), 1.93 (1H, m, 25-H), 2.22 (1H, dq-like, 20-H), 3.48 (1H, dd, J = 7.0, 9.5 Hz), 4.07 (1H, m) (26-H_2), 3.70 (1H, m, 3-H), 3.95 (1H, m, 2-H), 3.97, 4.98 (1H each, both m, 6′-H_2), 4.38 (1H, dd, J = 5.4, 11.8 Hz), 4.54 (1H, dd, J = 2.6, 11.8 Hz) (6‴-H_2), 4.80 (1H, d, J = 7.3 Hz, 1″-H), 4.81 (1H, d, J = 7.9 Hz, 1‴-H), 4.95 (1H, d, J = 7.6 Hz, 1′-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS m/z: 905 $(M-H)^-$, 773 $(M-C_5H_9O_4)^-$, 611 $(M-C_{11}H_{19}O_9)^-$. Positive-mode FAB-MS m/z: 929 $(M+Na)^+$.

Trigoneoside Ib (**2**): A white powder, $[\alpha]_D^{24}$ −41.5° (c = 0.37, pyridine). High-resolution negative-mode FAB-MS: Calcd for $C_{44}H_{73}O_{19}$ $(M-H)^-$: 905.4722; Found: 905.4783. IR (KBr) cm^{-1}: 3405, 2930, 1082, 1049. ^{1}H-NMR (pyridine-d_5) δ: 0.73, 0.87 (3H each, both s, 19, 18-H_3), 0.99 (3H, d, J = 6.7 Hz, 27-H_3), 1.05 (1H, m, 5-H), 1.32 (3H, d, J = 6.7 Hz, 21-H_3), 1.93 (1H, m, 25-H), 2.23 (1H, dq-like, 20-H), 3.63 (1H, dd, J = 6.1, 9.2 Hz), 3.95 (1H, m) (26-H_2), 3.70 (1H, m, 3-H), 3.96 (1H, m, 2-H), 3.98, 5.00 (1H each, both m, 6′-H_2), 4.80 (1H, d, J = 6.1 Hz, 1″-H), 4.81 (1H, d, J = 7.6 Hz, 1‴-H), 4.94 (1H, d, J = 7.6 Hz, 1′-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS m/z: 905 $(M-H)^-$, 773 $(M-C_5H_9O_4)^-$, 611 $(M-C_{11}H_{19}O_9)^-$. Positive-mode FAB-MS m/z: 929 $(M+Na)^+$.

Trigoneoside IIa (**3**): A white powder, $[\alpha]_D^{26}$ −47.4° (c = 1.77, pyridine). High-resolution positive-mode FAB-MS: Calcd for $C_{44}H_{74}NaO_{18}$ $(M+Na)^+$: 913.4773; Found: 913.4789. IR (KBr) cm^{-1}: 3400, 2928, 1078, 1040. ^{1}H-NMR (pyridine-d_5) δ: 0.85, 0.87 (3H each, both s, 19, 18-H_3), 1.03 (3H, d, J = 6.8 Hz, 27-H_3), 1.33 (3H, d, J = 6.7 Hz, 21-H_3), 1.93 (1H, m, 25-H), 1.98 (1H, m, 5-H), 2.24 (1H, dq-like, 20-H), 3.48 (1H, dd, J = 7.1, 9.5 Hz), 4.08 (1H, dd, J = 5.6, 9.5 Hz) (26-H_2), 4.35, 4.82 (1H each, both m, 6′-H_2), 4.41 (1H, m, 3-H), 4.80 (1H, d, J = 7.6 Hz, 1‴-H), 4.87 (1H, d, J = 7.6 Hz, 1′-H), 4.98 (1H, m, 16-H), 5.04 (1H, d, J = 7.7 Hz, 1″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS m/z: 889 $(M-H)^-$, 757 $(M-C_5H_9O_4)^-$, 727 $(M-C_6H_{11}O_5)^-$, 595 $(M-C_{11}H_{19}O_9)^-$. Positive-mode FAB-MS m/z: 913 $(M+Na)^+$.

Trigoneoside IIb (**4**): A white powder, $[\alpha]_D^{22}$ −45.3° (c = 1.68, pyridine). High-resolution positive-mode FAB-MS: Calcd for $C_{44}H_{74}NaO_{18}$ $(M+Na)^+$: 913.4773; Found: 913.4777. IR (KBr) cm^{-1}: 3409, 2930, 1078, 1042. ^{1}H-NMR (pyridine-d_5) δ: 0.85, 0.87 (3H each, both s, 19, 18-H_3), 0.99 (3H, d, J = 6.7 Hz, 27-H_3), 1.35 (3H, d, J = 7.0 Hz, 21-H_3), 1.93 (1H, m, 25-H), 1.98 (1H, m, 5-H), 2.25 (1H, m, 20-H), 3.62 (1H,

dd, $J=6.1$, 9.5 Hz), 3.94 (1H, dd, $J=7.0$, 9.5 Hz) (26-H_2), 4.37 (1H, dd, $J=5.2$, 11.3 Hz), 4.81 (1H, dd, $J=1.8$, 11.3 Hz) (6′-H_2), 4.42 (1H, m, 3-H), 4.80 (1H, d, $J=7.7$ Hz, 1‴-H), 4.87 (1H, d, $J=7.7$ Hz, 1′-H), 5.00 (1H, ddd-like, 16-H), 5.04 (1H, d, $J=7.3$ Hz, 1″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS *m/z*: 889 $(M-H)^-$, 757 $(M-C_5H_9O_4)^-$, 727 $(M-C_6H_{11}O_5)^-$, 595 $(M-C_{11}H_{19}O_9)^-$. Positive-mode FAB-MS *m/z*: 913 $(M+Na)^+$.

Trigoneoside IIIa (**5**): A white powder, $[\alpha]_D^{27}$ −54.4° ($c=0.66$, pyridine). High-resolution positive-mode FAB-MS: Calcd for $C_{45}H_{76}NaO_{18}$ $(M+Na)^+$: 927.4929; Found: 927.4967. IR (KBr) cm^{-1}: 3413, 2932, 1076, 1048. ^{1}H-NMR (pyridine-d_5) δ: 0.88, 0.89 (3H each, both s, 19, 18-H_3), 1.03 (3H, d, $J=6.7$ Hz, 27-H_3), 1.32 (3H, d, $J=7.1$ Hz, 21-H_3), 1.77 (3H, d, $J=6.4$ Hz, 6″-H_3), 1.93 (1H, m, 25-H), 2.23 (1H, dq-like, 20-H), 3.49 (1H, dd, $J=7.0$, 9.5 Hz), 4.09 (1H, dd, $J=5.8$, 9.5 Hz) (26-H_2), 3.99 (1H, m, 3-H), 4.82 (1H, d, $J=7.7$ Hz, 1‴-H), 5.07 (1H, d, $J=7.4$ Hz, 1′-H), 6.36 (1H, brs, 1″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (*m/z*): 903 $(M-H)^-$, 757 $(M-C_6H_{11}O_4)^-$, 741 $(M-C_6H_{11}O_5)^-$, 595 $(M-C_{12}H_{21}O_9)^-$. Positive-mode FAB-MS *m/z*: 927 $(M+Na)^+$.

Trigoneoside IIIb (**6**): A white powder, $[\alpha]_D^{27}$ −21.7° ($c=0.06$, pyridine). High-resolution positive-mode FAB-MS: Calcd for $C_{45}H_{76}NaO_{18}$ $(M+Na)^+$: 927.4929; Found: 927.4910. IR (KBr) cm^{-1}: 3419, 2932, 1075, 1048. ^{1}H-NMR (pyridine-d_5) δ: 0.88, 0.89 (3H each, both s, 19, 18-H_3), 0.99 (3H, d, $J=6.7$ Hz, 27-H_3), 1.34 (3H, d, $J=7.0$ Hz, 21-H_3), 1.77 (3H, d, $J=6.4$ Hz, 6″-H_3), 1.93 (1H, m, 25-H), 2.24 (1H, dq-like, 20-H), 3.63 (1H, dd, $J=5.8$, 9.5 Hz), 3.95 (1H, dd, $J=7.3$, 9.5 Hz) (26-H_2), 4.01 (1H, m, 3-H), 4.83 (1H, d, $J=7.6$ Hz, 1‴-H), 5.08 (1H, d, $J=7.3$ Hz, 1′-H), 6.37 (1H, brs, 1″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS *m/z*: 903 $(M-H)^-$, 757 $(M-C_6H_{11}O_4)^-$, 741 $(M-C_6H_{11}O_5)^-$, 595 $(M-C_{12}H_{21}O_9)^-$. Positive-mode FAB-MS *m/z*: 927 $(M+Na)^+$.

Methanolysis of Trigoneosides Ia (1), Ib (2), IIa (3), IIb (4), IIIa (5), and IIIb (6) A solution of a trigoneoside (1 mg of **1**, **2**, **3**, **4**, **5**, or **6**) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i) and methyl xyloside (ii) from **1**, **2**, **3**, and **4**; i and the TMS derivative of methyl rhamnoside (iii) from **5** and **6**; GLC conditions: CBR-M25-025, 0.25 mm (i.d.) × 25 m capillary column, column temperature 140—280 °C, He flow rate 15 ml/min, t_R: i (17.9, 18.2, 19.3 min), ii (12.3, 12.7 min), iii (13.9, 14.3 min).

Acid Treatment of Trigoneoside Ia (1) Giving Neogitogenin (9) A solution of **1** (21.4 mg) in 2 N HCl–dioxane (1 : 1, v/v, 2 ml) was heated under reflux for 1 h, then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$. After work-up of the AcOEt extract in a usual manner, the crude product was purified by normal-phase silica-gel column chromatography [1 g, 1) $CHCl_3$–MeOH (20 : 1 → 10 : 1), 2) *n*-hexane–acetone (2 : 1)] and HPLC [YMC-Pack SIL (250 × 4.6 mm i.d.), $CHCl_3$–MeOH (15 : 1)] to give neogitogenin (**9**, 1.0 mg, 9.8%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H-NMR data with reported values.[9]

Acid Treatment of Trigoneoside Ib (2) Giving Gitogenin (10) A solution of **2** (32.0 mg) in 2 N HCl–dioxane (1 : 1, v/v, 2 ml) was heated under reflux for 1 h, then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$. After work-up of the AcOEt extract in a usual manner, the crude product was purified by normal-phase silica-gel column chromatography [1 g, $CHCl_3$–MeOH (20 : 1 → 10 : 1)] to give gitogenin (**10**, 2.9 mg, 19.0%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H-NMR data with reported values.[12]

Acid Treatment of Trigoneoside IIa (3) Giving Sarsasapogenin (11) A solution of **3** (15.2 mg) in 2 N HCl–50% aqueous EtOH (1 : 1, v/v, 1.5 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Amberlite IRA-93ZU (OH^- form) and filtered. After removal of the solvent *in vacuo* from the filtrate, the product was subjected to reversed-phase silica-gel column chromatography [1 g, MeOH–H_2O (80 : 20, v/v) → MeOH] and HPLC [YMC-Pack SIL (250 × 4.6 mm i.d.), $CHCl_3$–MeOH (50 : 1)] to give sarsasapogenin (**11**, 5.0 mg, 70.4%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H-NMR data with reported values.[13]

Acid Treatment of Trigoneoside IIb (4) Giving Smilagenin (12) A solution of **4** (15.0 mg) in 2 N HCl–50% aqueous EtOH (1 : 1, v/v, 1.5 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Amberlite IRA-93ZU (OH^- form) and filtered. After removal of the solvent *in vacuo* from the filtrate, the product was subjected to reversed-phase silica-gel column chromatography [1 g, MeOH–H_2O (80 : 20, v/v) → MeOH] to give smilagenin (**12**, 5.8 mg, 82.7%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H-NMR data with reported values.[14]

Acid Treatment of Trigoneoside IIIa (5) Giving Neotigogenin (13) A solution of **5** (10.5 mg) in 2 N HCl–50% aqueous EtOH (1 : 1, v/v, 1.0 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Amberlite IRA-93ZU (OH^- form) and filtered. After removal of the solvent *in vacuo* from the filtrate, the product was subjected to reversed-phase silica-gel column chromatography [1 g, MeOH–H_2O (80 : 20, v/v) → MeOH] and HPLC [YMC-Pack SIL (250 × 4.6 mm i.d.), $CHCl_3$–MeOH (30 : 1)] to give neotigogenin (**13**, 1.6 mg, 33.1%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR data with reported values.[9]

Acid Treatment of Trigoneoside IIIb (6) Giving Tigogenin (14) A solution of **6** (10.3 mg) in 2 N HCl–50% aqueous EtOH (1 : 1, v/v, 1.0 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Amberlite IRA-93ZU (OH^- form) and filtered. After removal of the solvent *in vacuo* from the filtrate, the product was subjected to reversed-phase silica-gel column chromatography [1 g, MeOH–H_2O (80 : 20, v/v) → MeOH] to give tigogenin (**14**, 2.8 mg, 57.9%), which was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR data with reported values.[15]

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