MEDICINAL FOODSTUFFS. VIII.[1] FENUGREEK SEED. (2) : STRUCTURES OF SIX NEW FUROSTANOL SAPONINS, TRIGONEOSIDES IVa, Va, Vb, VI, VIIb, AND VIIIb, FROM THE SEEDS OF INDIAN *TRIGONELLA FOENUM-GRAECUM* L.†

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Abstract — Following the characterization of trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, seven new steroidal saponins called trigoneosides IVa, Va, Vb, VI, VIIb, VIIIb, and IX were isolated from a medicinal foodstuff fenugreek seed, the seeds of *Trigonella foenum-graecum* L. (Leguminosae) originating from India. The structures of trigoneosides IVa, Va, Vb, VI, VIIb, and VIIIb were elucidated on the basis of chemical and physicochemical evidence.

In the previous paper,[2] we reported the isolation of six furostanol saponins, trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, from a medicinal foodstuff fenugreek seed ("Koroha" in Japanese), the seeds of *Trigonella foenum-graecum* L. (Leguminosae) originating from India and their structure elucidations. In a continuing study, we have isolated seven new furostanol saponins called trigoneosides IVa (**1**), Va (**2**), Vb (**3**), VI (**4**), VIIb (**5**), VIIIb (**6**), and IX from the polar fraction of the glycoside fraction from Indian fenugreek seed together with three known furostanol saponins, compound C (**7**),[3] glycoside F (**8**),[4] and trigonelloside C (**9**).[5] This paper offers the isolation of the polar saponins and the structure elucidations of trigoneosides IVa (**1**), Va (**2**), Vb (**3**), VI (**4**), VIIb (**5**), and VIIIb (**6**) on the basis of chemical and physicochemical evidence.[6]

Trigoneoside IVa (1)

Trigoneoside IVa (**1**) was isolated as a white powder and was deduced to possess a furostanol structure by TLC examination using the Ehrlich reagent.[7] The IR spectrum of **1** showed strong absorption bands at 3410, 1072, and 1052 cm^{-1} suggestive of oligoglycosidic structure. The negative-ion and positive-ion FABMS of **1** showed quasimolecular ions at *m/z* 1063 $(M-H)^-$ and *m/z* 1087 $(M+Na)^+$, respectively, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{51}H_{84}O_{23}$. Methanolysis of **1** with 9% hydrogen chloride in dry methanol liberated yamogenin (**10**)[5b,8] having the 25*S*-configuration as the sapogenol and the methyl glycosides of glucose and rhamnose in a *ca.* 3 : 1 ratio.[9] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR analytical methods,[10] indicated the presence of the furost-5-ene moiety [δ 0.90 (s, 18-H_3), 1.03 (d, *J*=6.7 Hz, 27-H_3), 1.06 (s, 19-H_3), 1.33 (d, *J*=7.1 Hz, 21-H_3), 2.24 (dq, *J*=6.9, 6.9 Hz, 20-H), 5.29 (d-like, 6-H)], 3-*O*-β-D-glucopyranosyl part [δ 4.95 (d, *J*=9.0 Hz, 1'-H)], 2'-*O*-α-L-rhamnopyranosyl part [δ 1.77 (d, *J*=6.4 Hz, 6''-H_3), 6.26 (br s, 1''-H)], 4'-*O*-β-D-glucopyranosyl part [δ 5.14 (d, *J*=8.0 Hz, 1'''-H)], and 26-*O*-β-D-

† Dedicated to Dr. Koji Nakanishi, Professor Columbia University, in the celebration of his 75th birthday.

glucopyranosyl part [δ 4.82 (d, *J*=8.0 Hz, 1''''-H)]. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra of **1** were shown to be superimposable on those of glycoside F (**8**), except for the proton signals due to the 26-methylene group [δ 3.49 (dd, *J*=7.1, 9.5 Hz), 4.09 (dd, *J*=6.1, 9.5 Hz)], which were very similar to those of 26-*O*-β-D-glucopyranosyl-25(*S*)-furostanol glycosides such as trigoneosides Ia, IIa, and IIIa.[2] The 3,26-bisdesmoside structure of **1** was clarified by a heteronuclear multiple bond correlation (HMBC) experiment. Thus, long-range correlations were observed between the 1''''-proton and the 4'-carbon, between the 1''-proton and the 2'-carbon, between the 1'-proton and 3-carbon, and between the 1''''-proton and the 26-carbon. Consequently, the structure of trigoneoside IVa was determined to be as 26-*O*-β-D-glucopyranosyl-(25*S*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-[α-L-rhamnopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranoside (**1**).

Trigoneosides Va (2) and Vb (3)

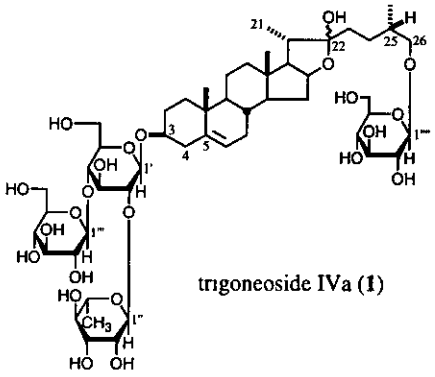
Trigoneosides Va (**2**) and Vb (**3**) were also isolated as a white powder and were deduced to possess a furostanol structure based on the positive Ehrlich test. The IR spectra of **2** and **3** showed strong absorption bands due to the oligoglycoside structure. Trigoneosides Va (**2**) and Vb (**3**) were found to have the same molecular formula $C_{68}H_{112}O_{37}$, which was determined from their negative-ion and positive-ion FABMS and by high-resolution MS measurement. Thus, in the negative-ion FABMS of **2** and **3**, the quasimolecular ion peak was observed at *m/z* 1519 $(M-H)^-$, while their positive-ion FABMS showed the quasimolecular ion peak at *m/z* 1543 $(M+Na)^+$. Furthermore, fragment ion peaks were observed at *m/z* 1387 $(M-C_5H_9O_4)^-$, *m/z* 1357 $(M-C_6H_{11}O_5)^-$, *m/z* 1063 $(M-C_{17}H_{29}O_{14})^-$, and *m/z* 901 $(M-C_{23}H_{39}O_{19})^-$ in the negative-ion FABMS of **2** and **3**. Methanolysis of **2** and **3** liberated yamogenin (**10**) and diosgenin (**11**)[5b,8] as the sapogenol, respectively, together with their common methyl glycosides of glucose, rhamnose, and xylose in a *ca.* 5 : 1 : 1 ratio.[9] Acid hydrolysis of **3** with 5% aqueous sulfuric acid-1,4-dioxane (1 : 1, v/v) furnished D-glucose, L-rhamnose, and D-xylose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[11]

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[10] of **2** showed signals ascribable to the 25*S*-furost-5-ene part [δ 0.90 (s, 18-H$_3$), 1.04 (d, *J*=6.7 Hz, 27-H$_3$), 1.07 (s, 19-H$_3$), 1.33 (d, *J*=6.7 Hz, 21-H$_3$), 2.24 (dq, *J*=7.0, 7.0 Hz, 20-H), 3.50 (dd, *J*=7.1, 9.2 Hz), 4.09 (m, 26-H$_2$), 5.29 (br s, 6-H)], five β-D-glucopyranosyl moieties [δ 4.83 (d, *J*=7.7 Hz, 1''''''-H), 4.95 (d-like, 1'-H), 5.08 (d-like, 1'''-H), 5.24 (d, *J*=8.0 Hz, 1''''-H), 5.28 (d, *J*=8.0 Hz, 1'''''-H), α-L-rhamnopyranosyl moiety [δ 1.78 (d, *J*=6.1 Hz, 6''-H$_3$), 6.25 (br s, 1''-H)], and β-D-xylopyranosyl moiety [δ 5.33 (d, *J*=7.6 Hz, 1'''''-H)]. The carbon signals of the 26-*O*-β-D-glucopyranosyl-(25*S*)-furost-5-ene-3β,22ξ,26-triol moiety in the ^{13}C-NMR spectrum of **2** were found to be superimposable on those of trigoneoside IVa (**1**) and compound C (**7**). The HMBC experiment of **2** showed long-range correlations between the following protons and carbons : 1''''''-H and 6''''-C, 1''''-H and 4''''-C, 1''''-H and 3'''-C, 1'''-H and 4'-C, 1''-H and 2'-C, 1'-H and 3-C, 1''''''-H and 26-C, so that the 3,26-bisdesmoside structure of **2** was characterized. On the basis of this evidence, the structure of trigoneoside Va was formulated as 26-*O*-β-D-glucopyranosyl-(25*S*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl (1→2)} {[β-D-xylopyranosyl (1→4)] [β-D-glucopyranosyl (1→6)]-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→4)}-β-D-glucopyranoside (**2**).

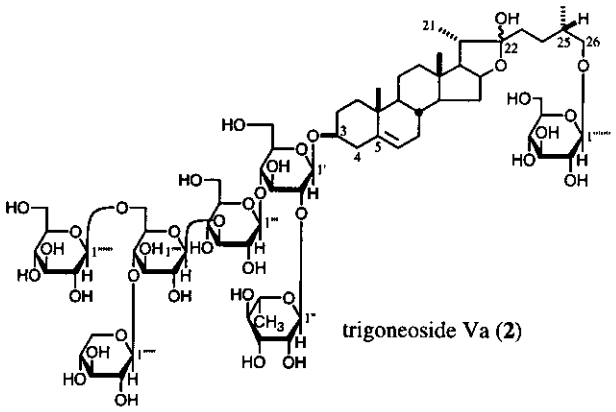
The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra[10] of **3** were superimposable to those of **2**, except for the proton signals due to the 26-methylene [δ 3.62 (dd, *J*=6.1, 9 8 Hz), 3.92 (m)], which showed the 25*R*-configuration.[2] The HMBC experiment of **3** showed long-range correlations between the same protons and carbons as those of **2**. Consequently, the structure of trigoneoside Vb was determined to be the 25*R*-isomer (**3**) of trigoneoside Va (**2**).

Trigoneoside VI (4), VIIb (5), and VIIIb (6)

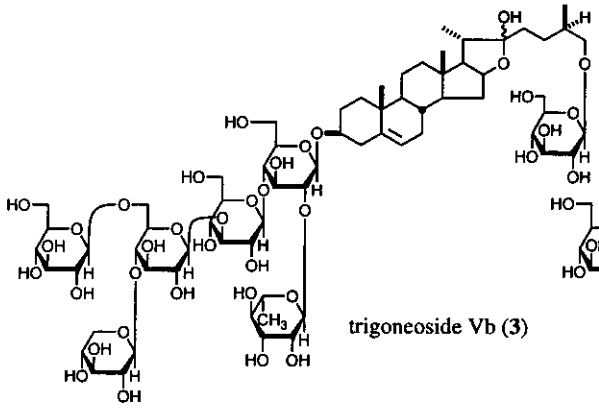
Trigoneosides VI (**4**), VIIb (**5**), and VIIIb (**6**), isolated as a white powder, were positive in the Ehrlich test. Methanolysis of **4** and **6** liberated sceptrumgenin (**12**)[12] and tigogenin (**13**), respectively, together with methyl glycosides of glucose, rhamnose, and xylose in a *ca.* 5 : 1 : 1 ratio,[9] while **5** liberated diosgenin (**11**) and methyl glycosides of glucose, rhamnose, and xylose



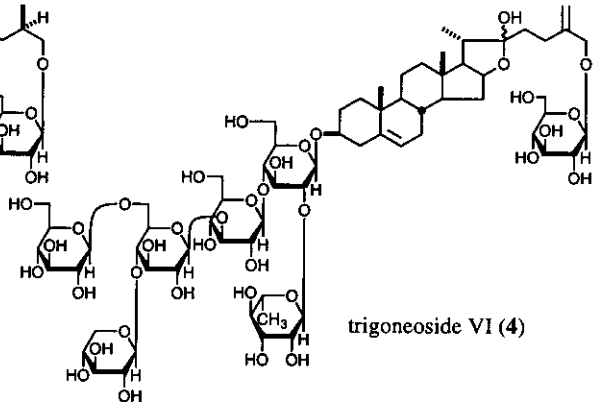
trigoneoside IVa (**1**)



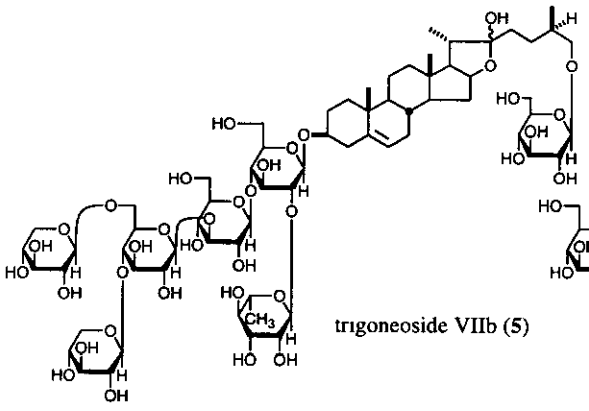
trigoneoside Va (**2**)



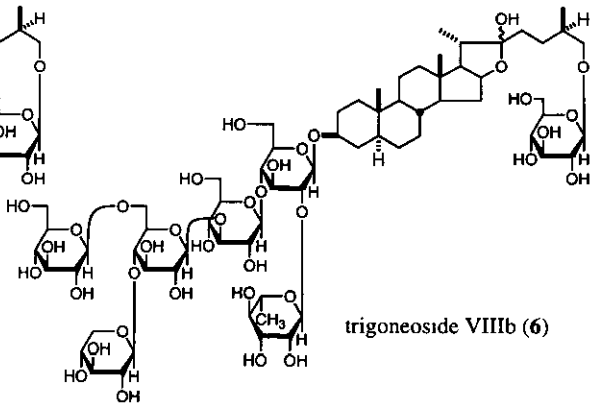
trigoneoside Vb (**3**)



trigoneoside VI (**4**)

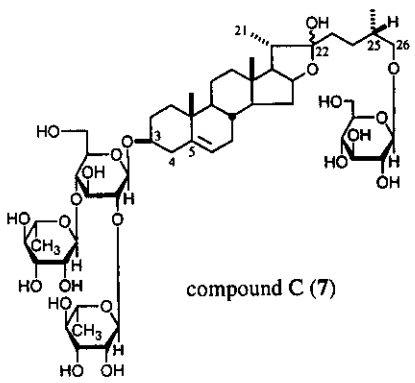


trigoneoside VIIb (**5**)

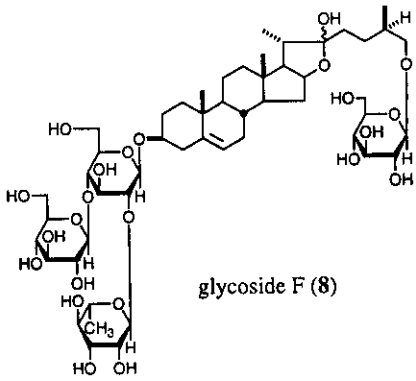


trigoneoside VIIIb (**6**)

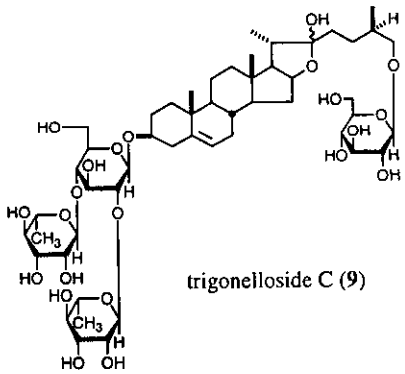
Scheme 1



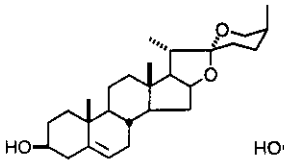
compound C (**7**)



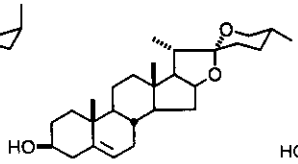
glycoside F (**8**)



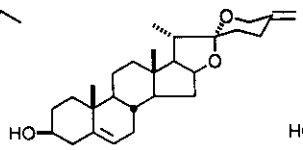
trigonelloside C (**9**)



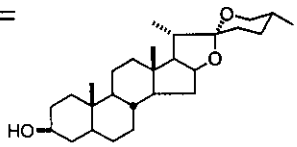
yamogenin (**10**)



diosgenin (**11**)



sceptrumgenin (**12**)



tigogenin (**13**)

Scheme 2

in a *ca.* 4 . 1 : 2 ratio[9] by the methanolysis. The molecular formula $C_{68}H_{110}O_{37}$ of **4** was determined from the negative-ion and positive-ion FABMS and by high-resolution MS measurement. In the positive-ion FABMS of **4**, the quasimolecular ion peak was observed at *m/z* 1541 $(M+Na)^+$, while the negative-ion FABMS of **4** showed the quasimolecular ion peak at *m/z* 1517 $(M-H)^-$ in addition to fragment ion peaks at *m/z* 1385 $(M-C_5H_9O_4)^-$, *m/z* 1355 $(M-C_6H_{11}O_5)^-$, *m/z* 1061 $(M-C_{17}H_{29}O_{14})^-$, and *m/z* 899 $(M-C_{23}H_{89}O_{19})^-$. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[10] of **4** indicated the presence of the furost-5,25 (27)-diene part [δ 0.89 (s, 18-H_3), 1.06 (s, 19-H_3), 1 33 (d, *J*=6.7 Hz, 21-H_3), 4.37, 4.62 (both

Table 1. ^{13}C-NMR Data of Trigoneosides IVa (**1**), Va (**2**), Vb (**3**), VI (**4**), VIIb (**5**), and VIIIb (**6**)

	1	**2**	**3**	**4**	**5**	**6**		**1**	**2**	**3**	**4**	**5**	**6**
C-1	37.5	37.5	37.5	37.4	37.6	37.2	2"	72.4	72.4	72.4	72.3	72.4	72.4
C-2	30.1	30.2	30.1	30.1	30.2	29.9	3"	72.7	72.8	72.7	72.7	72.8	72.8
C-3	78.1	78.2	78.2	78.2	78.3	77.2	4"	74.1	74.1	74.1	74.0	74.2	74.1
C-4	38.9	38.9	38.9	38.9	39.0	34.4	5"	69.5	69.5	69.4	69.4	69.4	69.4
C-5	140.7	140.8	140.8	140.7	140.9	44.6	6"	18.7	18.7	18.6	18.6	18.6	18.7
C-6	121.8	121.8	121.8	121.8	121.8	29.0	Glc-1"'	105.2	104.5	104.5	104.4	104.5	104.6
C-7	32.3	32.4	32.3	32.3	32.4	32.5	2"'	75.0	73.8	73.7	73.7	73.7	73.8
C-8	31.7	31.7	31.7	31.6	31.8	35.3	3"'	78.4	88.1	88.1	88.0	88.5	88.1
C-9	50.3	50.4	50.3	50.3	50.5	54.5	4"'	71.2	69.1	69.1	69.0	69.1	69.1
C-10	37.1	37.1	37.1	37.1	37.2	35.9	5"'	78.2	77.9	77.8	77.8	77.9	77.9
C-11	21.1	21.1	21.1	21.1	21.2	21.3	6"'	61.8	61.4	61.4	61.4	61.6	61.6
C-12	39.9	39.9	39.9	39.9	40.0	40.2	Glc-1""		105.4	105.3	105.3	105.5	105.4
C-13	40.8	40.8	40.8	40.7	40.8	41.1	2""		75.1	75.0	75.0	75.2	75.1
C-14	56.6	56.6	56.6	56.5	56.7	56.4	3""		76.1	76.1	76.0	76.1	76.1
C-15	32.5	32.5	32.5	32.4	32.5	32.4	4""		80.0	80.0	79.9	80.1	80.0
C-16	81.1	81.1	81.1	81.1	81.1	81.1	5""		75.3	75.2	75.1	75.4	75.3
C-17	63.8	63.8	63.8	63.7	63.9	64.0	6""		68.4	68.3	68.3	68.5	68.4
C-18	16.5	16.5	16.4	16.4	16.5	16.7	Xyl-1""		105.3	105.2	105.2	105.2	105.3
C-19	19.4	19.4	19.4	19.3	19.4	12.4	2""		74.9	74.8	74.8	74.8	74.9
C-20	40.7	40.7	40.7	40.6	40.7	40.7	3""		78.4	78.3	78.2	78.2	78.4
C-21	16.5	16.5	16.5	16.3	16.4	16.5	4""		70.9	70.8	70.8	70.9	70.9
C-22	110.7	110.7	110.7	110.3	110.7	110.6	5""		67.3	67.3	67.2	67.3	67.3
C-23	37.1	37.2	37.1	37.9	37.2	37.2	Glc (or Xyl)-1""		105.0	104.9	104.8	105.5	105.0
C-24	28.3	28.3	28.3	28.3	28.4	28.4	2""		75.4	75.3	75.3	75.1	75.4
C-25	34.4	34.4	34.3	147.2	34.3	34.3	3""		78.3	78.2	78.1	78.4	78.3
C-26	75.4	75.4	75.3	72.0	75.3	75.3	4""		71.5	71.5	71.5	71.7	71.5
C-27	17.4	17.5	17.5	110.7	17.5	17.5	5""		78.3	78.2	78.1	67.1	78.3
Glc-1'	100.0	99.9	99 9	99.9	100.1	99.5	6""		61.6	61.6	61.6		61.6
2'	77.3	77.2	77.2	77.2	77.3	77.5	Glc(26)-1""	105.1	105.1	104.9	103.8	104.9	104.9
3'	76.2	76.3	76.2	76.1	76.2	76.3	2""	75.2	75.2	75.1	75.2	75.2	75.2
4'	81.9	81.4	81.4	81.3	81.6	81.6	3""	78.6	78.6	78.5	78.5	78.6	78.6
5'	77.7	77.6	77.6	77.5	77.6	77.6	4""	71.6	71.7	71.7	71.6	71.8	71.7
6'	62.1	62.6	62.6	62.5	61.7	62.6	5""	78.4	78.5	78.4	78.4	78.4	78.5
Rha-1"	101.8	101.8	101.8	101.7	101.8	101.9	6""	62.8	62.8	62.8	62.7	62.9	62.8

125 MHz, pyridine-d_5

m, 26-H_2), 5.06 (m), 5.34 (br s, 27-H_2), 5.30 (br s, 6-H)], five β-D-glucopyranosyl moieties [δ 4.94 (d-like, 1'-H), 5.07 (d, *J*=8.6 Hz, 1'''-H), 5.21 (d, *J*=7.0 Hz, 1''''-H), 5.22 (d, *J*=7.0 Hz, 1'''''-H), 4.89 (d, *J*=7.9 Hz, 1''''''-H)], α-L-rhamnopyranosyl moiety [δ 1.77 (d, *J*=6.1 Hz, 6''-H_3), 6.22 (br s, 1''-H)], and β-D-xylopyranosyl moiety [δ 5.27 (d, *J*=7.7 Hz, 1'''''-H)]. The carbon signals of the saccharide parts in the ^{13}C-NMR spectrum of **4** were very similar to those of **2** and **3**. Furthermore, in the HMBC experiment of **4**, long-range correlations were observed between the following protons and carbons : 1'''''-H and 6'''''-C, 1'''''-H and 4'''''-C, 1''''-H and 3'''-C, 1'''-H and 4'-C, 1''-H and 2'-C, 1'-H and 3-C, 1''''''-H and 26-C. Those finding led us to formulate the structure of trigoneoside VI as 26-*O*-β-D-glucopyranosyl-furost-5,25(27)-diene-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl (1→2)} {[β-D-xylopyranosyl (1→4)] [β-D-glucopyranosyl (1→6)]-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→4)]}-β-D-glucopyranoside (**4**).

The molecular formula $C_{67}H_{110}O_{36}$ of trigoneoside VIIb (**5**) was determined from the quasimolecular ion peaks in their negative-ion [*m/z* 1489 $(M-H)^-$, *m/z* 1357 $(M-C_5H_9O_4)^-$, *m/z* 1327 $(M-C_6H_{11}O_5)^-$, *m/z* 1063 $(M-C_{16}H_{27}O_{13})^-$] and positive-ion [*m/z* 1513 $(M+Na)^+$] FABMS and by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) spectrum[10] of **5** showed signals due to the 25*R*-furost-5-ene part [δ 0.90 (s, 18-H_3), 0.99 (d, *J*=6.8 Hz, 27-H_3), 1.05 (s, 19-H_3), 1.32 (d, *J*=7.0 Hz, 21-H_3), 3.62 (dd, *J*=5.8, 9.5 Hz), 3.92 (m, 26-H_2), 3.84 (m, 3-H), 5.30 (br s, 6-H)], four β-D-glucopyranosyl moieties [δ 4.79 (d, *J*=8.0 Hz, 1''''''-H), 4.91 (d-like, 1'-H), 5.02 (d, *J*=6.4 Hz, 1'''-H), 5.04 (d, *J*=7.3 Hz, 1''''-H)], α-L-rhamnopyranosyl moiety [δ 1.74 (d, *J*=6.5 Hz, 6''-H_3), 6.17 (br s, 1''-H)], and two β-D-xylopyranosyl moieties [δ 5.15 (d, *J*=8.6 Hz, 1'''''-H), 5.25 (d, *J*=7.7 Hz, 1''''''-H)]. The carbon signals of **5** in the ^{13}C-NMR (Table 1) spectrum[10] was very similar to those of **3**, except for the signals due to terminal β-D-xylopyranosyl moiety. The HMBC experiment of **5** showed long-range correlations between the following protons and carbons : 1'''''-H and 6''''-C, 1''''-H and 4''''-C, 1''''-H and 3'''-C, 1'''-H and 4'-C, 1''-H and 2'-C, 1'-H and 3-C, 1''''''-H and 26-C. On the basis of this evidence, the structure of trigoneoside VIIb (**5**) was clarified as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl (1→2)} {[β-D-xylopyranosyl (1→4)] [β-D-xylopyranosyl (1→6)]-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→4)}-β-D-glucopyranoside (**5**).

Trigoneoside VIIIb (**6**) was found to have the molecular formula $C_{68}H_{114}O_{37}$, which was determined from the quasimolecular ion peaks in the negative-ion [*m/z* 1521 $(M-H)^-$, *m/z* 1389 $(M-C_5H_9O_4)^-$, *m/z* 1359 $(M-C_6H_{11}O_4)^-$, *m/z* 1065 $(M-C_{17}H_{29}O_{14})^-$, *m/z* 903 $(M-C_{23}H_{39}O_{19})^-$] and positive-ion [*m/z* 1545 $(M+Na)^+$] FABMS and by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) spectrum[10] of **6** indicated the presence of the 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostanol part [δ 0.87 (s, 19-H_3), 0.89 (s, 18-H_3), 0.99 (d, *J*=6.4 Hz, 27-H_3), 1.34 (d, *J*=6.8 Hz, 21-H_3), 3.63 (dd, *J*=5.8, 9.2 Hz), 3.94 (m, 26-H_2), 3.92 (m, 3-H)], five β-D-glucopyranosyl moieties [δ 4.83 (d, *J*=8.0 Hz, 1''''''-H), 4.97 (d, *J*=7.0 Hz, 1'-H), 5.08 (d, *J*=7.7 Hz, 1'''-H), 5.24 (d, *J*=7.9 Hz, 1''''-H), 5.28 (d, *J*=7.9 Hz, 1'''''-H)], α-L-rhamnopyranosyl moiety [δ 1.76 (d, *J*=6.1 Hz, 6''-H_3), 6.22 (br s, 1''-H)], and β-D-xylopyranosyl moiety [δ 5.32 (d, *J*=7.9 Hz, 1'''''-H)]. The carbon signals due to the 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-3β,22ξ,26-triol part in the ^{13}C-NMR (Table 1) spectrum[10] of **6** were superimposable on those of trigoneoside IIIb,[2] whereas the carbon signals due to the oligoglycoside at the 3-position resembled to those of **2**, **3**, and **4**. Finally, the HMBC experiment of **6** led us to formulate the structure of trigoneoside VIIIb as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-3β,22ξ,26-triol 3-*O*-{α-L-rhamnopyranosyl (1→2)} {[β-D-xylopyranosyl (1→4)] [β-D-glucopyranosyl (1→6)]-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl (1→4)}-β-D-glucopyranoside (**6**).

EXPERIMENTAL

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper [13]

Isolation of Trigoneosides IVa (1), Va (2), Vb (3), VI (4), VIIb (5), VIIIb (6), and IX and Known Compounds (7, 8, 9)

Fractions 2-5 (1.6 g, 0.059%), 5 (36.2 g, 1.4%), 6 (11.5 g, 0.44%), and 7 (26.7 g, 1.02%) were obtained from the seeds of *Trigonella foenum-graecum* L. (10 kg, cultivated in India and purchased from Honso Pharmaceutical Co., Ltd., Nagoya) as reported previously.[1] Fractions 2-5 (1.6 g) were purified by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 x 20 mm i.d.), MeCN-H_2O (35 : 65, v/v)] to give trigoneoside IX (271 mg, 0.010%). Fraction 5 (36.2 g) was separated by HPLC [MeCN-H_2O (25 : 75, v/v)] to give trigoneoside IVa (**1**, 4339 mg, 0.16%), glycoside F (**8**, 8136 mg, 0.30%), compound C (**7**, 9492 mg, 0.35%), and trigonelloside C (**9**, 4068 mg, 0.15%). Fractions 6 (11.5 g) and 7 (26.7 g) were separated by HPLC [MeCN-H_2O (25 : 75, v/v)] to give trigoneosides Va (**2**, 6508 mg, 0.24%), Vb (**3**, 8949 mg, 0.33%), and VI (**4**, 1790 mg, 0.066%) and crude saponin fraction (2712 mg). Crude saponin fraction (2712 mg) was separated by HPLC [1) YMC-Pack ODS-AL (YMC Co , Ltd., 250 x 20 mm i.d.), MeCN-H_2O (25 : 75, v/v); 2) YMC-Pack ODS-A (YMC Co., Ltd., 250 x 20 mm i.d.), MeOH-H_2O (60 : 40, v/v)] to give 22-methoxytrigoneosides VIIb (461 mg, 0.017%) and VIIIb (407 mg, 0.015%). 22-Methoxytrigoneosides VIIb (461 mg) and VIIIb (407 mg) in MeCN-H_2O (10 mL, 1.: 1, v/v) were stirred under reflux for 3 h to give trigoneosides VIIb (quant.) and VIIIb (quant.). The known compounds (**7-9**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[3-5]

Trigoneoside IVa (**1**) : a white powder, $[\alpha]_D^{28}$ -56.5° (*c*=0.8, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{51}H_{84}O_{23}Na$ $(M+Na)^+$: 1087.5301; Found : 1087.5320. IR (KBr, cm^{-1}) : 3410, 2934, 1072, 1052. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.90 (3H, s, 18-H_3), 1.03 (3H, d, *J*=6.7 Hz, 27-H_3), 1.06 (3H, s, 19-H_3), 1.33 (3H, d, *J*=7.1 Hz, 21-H_3), 1.77 (3H, d, *J*=6.4 Hz, Rha-6-H_3), 2.24 (1H, dq, *J*=6.9, 6.9 Hz, 20-H), 3.49 (1H, dd, *J*=7.1, 9.5 Hz), 4.09 (1H, dd, *J*=6.1, 9.5 Hz, 26-H_2), 3.88 (1H, m, 3-H), 4.82 (1H, d, *J*=8.0 Hz, Glc-1'''-H), 4.95 (1H, d, *J*=9.0 Hz, Glc-1'-H), 5.14 (1H, d, *J*=8.0 Hz, Glc-1'''-H), 5.29 (1H, d-like, 6-H), 6.26 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS (*m/z*) : 1063 $(M-H)^-$. Positive-ion FABMS (*m/z*) : 1087 $(M+Na)^+$.

Trigoneoside Va (**2**) : a white powder, $[\alpha]_D^{26}$ -53.8° (*c*=0.3, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{68}H_{112}O_{37}Na$ $(M+Na)^+$: 1543.6780; Found : 1543.6730. IR (KBr, cm^{-1}) : 3400, 2931, 1159, 1074, 1044. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.90 (3H, s, 18-H_3), 1.04 (3H, d, *J*=6.7 Hz, 27-H_3), 1.07 (3H, s, 19-H_3), 1.33 (3H, d, *J*=6.7 Hz, 21-H_3), 1.78 (3H, d, *J*=6.1 Hz, Rha-6"-H_3), 2.24 (1H, dq, *J*=7.0, 7.0 Hz, 20-H), 3.50 (1H, dd, J=7.1, 9.2 Hz), 4.09 (1H, m, 26-H_2), 3.88 (1H, m, 3-H), 4.83 (1H, d, *J*=7.7 Hz, Glc-1''''''-H), 4.95 (1H, d-like, Glc-1'-H), 5.08 (1H, d-like, Glc-1'''-H), 5.24 (1H, d, *J*=8.0 Hz, Glc-1''''-H), 5.28 (1H, d, *J*=8.0 Hz, Glc-1''''''-H), 5.29 (1H, br s, 6-H), 5.33 (1H, d, *J*=7.6 Hz, Xyl-1''''-H), 6.25 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS (*m/z*) : 1519 $(M-H)^-$, 1387 $(M-C_5H_9O_4)^-$, 1357 $(M-C_6H_{11}O_5)^-$, 1063 $(M-C_{17}H_{29}O_{14})^-$, 901 $(M-C_{23}H_{39}O_{19})^-$. Positive-ion FABMS (*m/z*) : 1543 $(M+Na)^+$.

Trigoneoside Vb (**3**) : a white powder, $[\alpha]_D^{24}$ -55.6° (*c*=0.5, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{68}H_{112}O_{37}Na$ $(M+Na)^+$: 1543.6780; Found : 1543.6868. IR (KBr, cm^{-1}) : 3400, 2934, 1159, 1074, 1050. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.90 (3H, s, 18-H_3), 0.99 (3H, d, *J*=6.4 Hz, 27-H_3), 1.06 (3H, s, 19-H_3), 1.34 (3H, d, *J*=6.8 Hz, 21-H_3), 1.77 (3H, d, *J*=6.4 Hz, Rha-6"-H_3), 2.25 (1H, dq, *J*=7.0, 7.0 Hz, 20-H), 3.62 (1H, dd, *J*=6.1, 9.8 Hz), 3.92 (1H, m, 26-H_2), 3.88 (1H, m, 3-H), 4.81 (1H, d, *J*=7.9 Hz, Glc-1''''''-H), 4.95 (1H, d, *J*=6.7 Hz, Glc-1'-H), 5.08 (1H, d, *J*=8.6 Hz, Glc-1'''-H), 5.22 (1H, d, *J*=9.2 Hz, Glc-1''''-H), 5.23 (1H, d, *J*=8.0 Hz, Glc-1''''''-H), 5.28 (1H, d, *J*=8.0 Hz, Xyl-1''''''-H), 5.30 (1H, br s, 6-H), 6.22 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS

(*m/z*) : 1519 (M-H)$^-$, 1387 (M-$C_5H_9O_4$)$^-$, 1357 (M-$C_6H_{11}O_5$)$^-$, 1063 (M-$C_{17}H_{29}O_{14}$)$^-$, 901 (M-$C_{23}H_{39}O_{19}$)$^-$. Positive-ion FABMS (*m/z*) : 1543 (M+Na)$^+$.

Trigoneoside VI (**4**) : a white powder, $[\alpha]_D^{26}$ -54.7° (*c*=0.3, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{68}H_{110}O_{37}Na$ (M+Na)$^+$: 1541.6623; Found : 1541.6667. IR (KBr, cm^{-1}) : 3404, 2932, 1655, 1159, 1073, 1044. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.89 (3H, s, 18-H$_3$), 1.06 (3H, s, 19-H$_3$), 1.33 (3H, d, *J*=6.7 Hz, 21-H$_3$), 1.77 (3H, d, *J*=6.1 Hz, Rha-6"-H$_3$), 2.26 (1H, m, 20-H), 3.87 (1H, m, 3-H), 4.37, 4.62 (1H each, both m, 26-H$_2$), 4.89 (1H, d, *J*=7.9 Hz, Glc-1""""-H), 4.94 (1H, d-like, Glc-1'-H), 5.06 (1H, m), 5.34 (1H, br s, 27-H$_2$), 5.07 (1H, d, *J*=8.6 Hz, Glc-1"'-H), 5.21 (1H, d, *J*=7.0 Hz, Glc-1""-H), 5.22 (1H, d, *J*=7.0 Hz, Glc-1"""-H), 5.27 (1H, d, *J*=7.7 Hz, Xyl-1""""-H), 5.30 (1H, br s, 6-H), 6.22 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS (*m/z*) : 1517 (M-H)$^-$, 1385 (M-$C_5H_9O_4$)$^-$, 1355 (M-$C_6H_{11}O_5$)$^-$, 1061 (M-$C_{17}H_{29}O_{14}$)$^-$, 899 (M-$C_{23}H_{39}O_{19}$)$^-$. Positive-ion FABMS (*m/z*) : 1541 (M+Na)$^+$.

Trigoneoside VIIb (**5**) : a white powder, $[\alpha]_D^{27}$ -55.9° (*c*=1.7, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{67}H_{110}O_{36}Na$ (M+Na)$^+$: 1513.6675; Found : 1513.6665. IR (KBr, cm^{-1}) : 3409, 2930, 1161, 1071, 1044. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.90 (3H, s, 18-H$_3$), 0.99 (3H, d, *J*=6.8 Hz, 27-H$_3$), 1.05 (3H, s, 19-H$_3$), 1.32 (3H, d, *J*=7.0 Hz, 21-H$_3$), 1.74 (3H, d, *J*=6.5 Hz, Rha-6"-H$_3$), 2.24 (1H, dq, *J*=6.4, 6.4 Hz, 20-H), 3.62 (1H, dd, *J*=5.8, 9.5 Hz), 3.92 (1H, m, 26-H$_2$), 3.84 (1H, m, 3-H), 4.79 (1H, d, *J*=8.0 Hz, Glc-1""""-H), 4.91 (1H, d-like, Glc-1'-H), 5.02 (1H, d, *J*=6.4 Hz, Glc-1"'-H), 5.04 (1H, d, *J*=7.3 Hz, Glc-1""-H), 5.15 (1H, d, *J*=8.6 Hz, Xyl-1"""-H), 5.25 (1H, d, *J*=7.7 Hz, Xyl-1""""-H), 5.30 (1H, br s, 6-H), 6.17 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS (*m/z*) : 1489 (M-H)$^-$, 1357 (M-$C_5H_9O_4$)$^-$, 1327 (M-$C_6H_{11}O_5$)$^-$, 1063 (M-$C_{16}H_{27}O_{13}$)$^-$. Positive-ion FABMS (*m/z*) : 1513 (M+Na)$^+$.

Trigoneoside VIIIb (**6**) : a white powder, $[\alpha]_D^{23}$ -44.0° (*c*=0.3, pyridine). High-resolution positive-ion FABMS (*m/z*) : Calcd for $C_{68}H_{114}O_{37}Na$ (M+Na)$^+$: 1545.6937; Found : 1545.6923. IR (KBr, cm^{-1}) : 3416, 2932, 1073, 1044. ^{1}H-NMR (pyridine-d_5, 500 MHz, δ) : 0.87 (3H, s, 18-H$_3$), 0.89 (3H, s, 19-H$_3$), 0.99 (3H, d, *J*=6.4 Hz, 27-H$_3$), 1.34 (3H, d, *J*=6.8 Hz, 21-H$_3$), 1.76 (3H, d, *J*=6.1 Hz, Rha-6"-H$_3$), 2.24 (1H, dq, *J*=6.8, 6.8 Hz, 20-H), 3.63 (1H, dd, *J*=5.8, 9.2 Hz), 3.94 (1H, m, 26-H$_2$), 3.92 (1H, m, 3-H), 4.83 (1H, d, *J*=8.0 Hz, Glc-1""""-H), 4.97 (1H, d, *J*=7.0 Hz, Glc-1'-H), 5.08 (1H, d, *J*=7.7 Hz, Glc-1"'-H), 5.24 (1H, d, *J*=7.9 Hz, Glc-1""-H), 5.28 (1H, d, *J*=7.9 Hz, Glc-1"""-H), 5.32 (1H, d, *J*=7.9 Hz, Xyl-1""""-H), 6.22 (1H, br s, Rha-1"-H). ^{13}C-NMR (pyridine-d_5, 125 MHz, δ_C) : given in Table 1. Negative-ion FABMS (*m/z*) : 1521 (M-H)$^-$, 1389 (M-$C_5H_9O_4$)$^-$, 1359 (M-$C_6H_{11}O_5$)$^-$ 1065 (M-$C_{17}H_{29}O_{14}$)$^-$, 903 (M-$C_{23}H_{39}O_{19}$)$^-$. Positive-ion FABMS (*m/z*) : 1545 (M+Na)$^+$.

Methanolysis of Trigoneosides IVa (1), Va (2), Vb (3), VI (4), VIIb (5), and VIIIb (6)

A solution of trigoneosides (**1**-**6**, 5 mg each) in 9% HCl-MeOH (0.5 mL) was heated under reflux for 2.5 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the insoluble portion was removed by filtration. Each product, which was obtained from the filtrate by removal of the solvent under reduced pressure, was purified by reversed-phase silica gel column chromatography [Chromatorex ODS (Fuji Silysia Chemical Ltd., 0.5 g), MeOH-H_2O (80 : 20, v/v)] to give the sapogenol constituent [yamogenin (**10**, 0.6 mg, 30.5%) from **1**, **10** (0.7 mg, 50.8 %) from **2**, diosgenin (**11**, 1.0 mg, 50.8 %) from **3**, sceptrumgenin (**12**, 0.9 mg, 64.6%) from **4**, **11** (0.7 mg, 50.6 %) from **5**, tigogenin (**13**, 0.7 mg, 51.5%) from **6**]. **10**, **11**, **12**, and **13** was identified by comparison of melting point, $[\alpha]_D$, and ^{1}H-NMR data with reported values.[5b,8,11,14] The sugar composition of the product was analyzed by GLC. A solution of each product in pyridine (0.02

mL) was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.01 mL) for 1 h. The reaction solution was then subjected to GLC analysis to identify trimethylsilyl (TMS) derivatives of methyl glycosides [methyl glucoside (i) and methyl rhamnoside (ii) from **1**, i, ii, and methyl xyloside (iii) from **2**, **3**, **4**, **5**, and **6**]. GLC conditions : CBR-M25-025, 0.25 mm (i.d.) x 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 Hydrolysis of Trigoneoside Vb (3)

A solution of trigoneoside Vb (**3**, 2 mg) in 5% aqueous H_2SO_4-1,4-dioxane (1 mL, 1 : 1, v/v) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was separated on a Sep-Pack C_{18} cartridge column ($H_2O \rightarrow$MeOH). The H_2O eluate was concentrated under reduced pressure to give a residue, which was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 mL) and the mixture was left standing at 60 °C for 1 h. The reaction solution was then treated with BSTFA (0.01 mL) and the whole mixture was subjected to GLC analysis to identify the trimethylsilyl thiazolidine derivatives of D-glucose (i), L-rhamnose (ii), and D-xylose (iii). GLC conditions : column, Supelco SPB^{TM}-1, 0.25 mm (i.d.) x 30 m; column temperature, 230 °C; t_R, i, 24.2 min; ii, 15.4 min; iii, 13.8 min.

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