Hosenkosides F, G, H, I, J, and K, Novel Baccharane Glycosides from the Seeds of *Impatiens balsamina*

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Received January 28, 1994; accepted March 16, 1994

From the seeds of *Impatiens balsamina* we have isolated six novel baccharane glycosides, hosenkosides F—K. The structures of all isolates were determined by the use of two dimensional (2D) NMR techniques (^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), rotating frame Overhauser enhancement spectroscopy (ROESY), total correlation spectroscopy (TOCSY)) and chemical derivatization. Hosenkosides F, H and I are hosenkol B 3-*O*-sambubiosido-26-*O*-glucoside, 3-*O*-sambubioside and 3,26-*O*-diglucoside, respectively. Hosenkoside G is hosenkol C 3-*O*-sambubiosido-28-*O*-glucoside. Hosenkosides J and K are hosenkol A 3-*O*-sophoroside and 3-*O*-sophorosido-26-*O*-glucosyl-28-*O*-glucoside, respectively.

Keywords *Impatiens balsamina*; Balsaminaceae; baccharane glycoside; hosenkoside

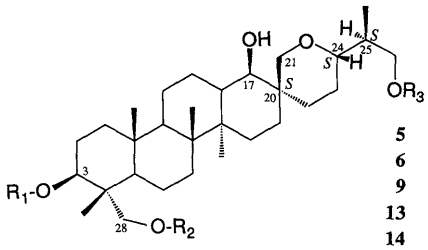
Impatiens balsamina L., an annual native to India, is now widely cultivated as an ornamental plant. The seeds have been used to treat difficult labor, to suppress puerperal pain, and to act as an emmenagogue, expectorant, and as an antidote for poisoning from fish in some Oriental countries.[1,2]

We have already reported the isolation and structure determination of five saponins, hosenkosides A—E[3] from the seeds of *I. balsamina*. Hosenkosides A and D are hosenkol A[3,4] 3-*O*-sophorosido-28-*O*-glucoside (**14**), and 3, 28-*O*-diglucoside (**15**), respectively. Hosenkosides B and E are hosenkol B 3-*O*-sophorosido-26-*O*-glucoside, and 3-*O*-sophoroside, respectively. Hosenkoside C is hosenkol C 3-*O*-sophorosido-28-*O*-glucoside. In this paper, we report the isolation and structural elucidation of six additional novel saponins, hosenkosides F—K (**1**—**6**), having a rare baccharane skeleton. Their structures were elucidated by chemical and spectral methods, two dimensional (2D)-NMR techniques being especially helpful.

The 50% MeOH extract of the seed of *Impatiens balsamina*, after treatment with *n*-BuOH, gave a saponin fraction. Repeated separation of the saponin fraction by ordinary-phase SiO_2 and reversed-phase (silanized SiO_2) furnished six new saponins hosenkosides, F (**1**), G (**2**), H (**3**), I (**4**), J (**5**) and K (**6**). ^{1}H–^{1}H Correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H–^{13}C COSY, total correlation spectroscopy (TOCSY), heteronuclear multiple-bond correlation (HMBC) and rotating frame Overhauser enhancement spectroscopy (ROESY) experiments led to the determination of the complete structures of **1**—**6**, inclusive of the sequence of the sugar moieties and the position of attachment of the sugar chains to the aglycon.

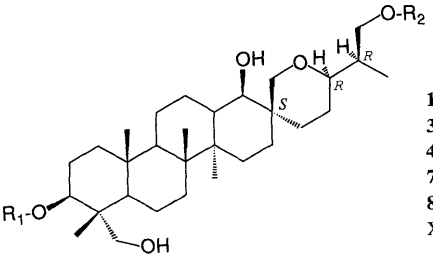
Hosenkoside H (**3**) was obtained as colorless needles. The negative FAB-MS of **3** showed ion peaks at *m/z* 785 $[M-H]^-$ and 653 $[M-C_5H_8O_4-H]^-$, suggesting the molecular formula, $C_{41}H_{70}O_{14}$. On acid hydrolysis, **3** afforded hosenkol B (**7**),[3] besides D-glucose and D-xylose in the ratio 1 : 1, confirmed by chiral detection in HPLC. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **3** indicated the presence of one β-D-glucopyranosyl unit [H-1′: δ 5.15 (d, J=7.8 Hz), C-1′: δ 104.2] and one β-D-xylopyranosyl unit [H-1″: δ 5.24 (d, J=6.8 Hz), C-1″: δ 107.1]. A crude cellulase treatment of **3** gave **7** and presapogenin I (**8**).

Presapogenin I (**8**), $[\alpha]_D^{20}$ +30.2° (pyridine) revealed a molecular ion peak at *m/z* 653 $[M-H]^-$ in the negative FAB-MS, suggesting that **8** was a monoglucoside. The C-3 signal in the ^{13}C-NMR spectrum of **8** appeared at lower field by 8.6 ppm than that of **7** because of the glycosylation shift,[5,6] demonstrating that a β-glucopy-



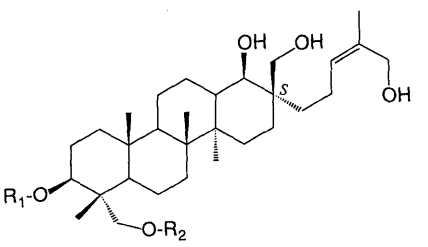
	R_1	R_2	R_3
5	-Glc^{2}Glc	-H	-H
6	-Glc^{2}Glc	-Glc	-Glc
9	-H	-H	-H
13	-Glc	-H	-H
14	-Glc^{2}Glc	-Glc	-H
15	-Glc	-Glc	-H

Glc=β-D-glucopyranosyl



	R_1	R_2
1	-Glc^{2}Xyl	-Glc
3	-Glc^{2}Xyl	-H
4	-Glc	-Glc
7	-H	-H
8	-Glc	-H

Xyl=β-D-xylopyranosyl



	R_1	R_2
2	-Glc^{2}Xyl	-Glc
10	-Glc	-Glc
11	-Glc	-H
12	-H	-H

ranosyl group is located at the C-3-OH of the aglycon. Therefore, **8** was determined to be hosenkol B 3-*O*-*β*-D-glucopyranoside. Comparison of the ^{13}C-NMR spectrum of **3** with that of **8** showed a glycosylation shift for the C-2 signal (+8.7 ppm) of glucosyl, demonstrating that a *β*-xylopyranosyl group is located at the C-2-OH of glucose. Therefore, **3** was formulated as hosenkol B 3-*O*-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranoside.

Hosenkoside F (**1**), one of the minor saponins, $[\alpha]_D^{20}$ +7.2° (pyridine) had the molecular formula $C_{47}H_{80}O_{19}\cdot H_2O$ based on the elementary analysis. On acid hydrolysis, **1** afforded **7**, besides D-glucose and D-xylose in the ratio 2:1. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **1** indicated the presence of two *β*-D-glucopyranosyl units [H-1′: *δ* 5.15(d, *J*=6.8 Hz), C-1′: *δ* 104.2; H-1‴: *δ* 4.87 (d, *J*=7.8 Hz), C-1‴: *δ* 105.0], and one *β*-D-xylopyranosyl unit [H-1″: *δ* 5.24 (d, *J*=6.4 Hz), C-1″: *δ* 107.1]. A crude cellulase treatment of **1** gave **3**, **7** and **8**. A ^{13}C-NMR spectral comparison of **1** with **3** showed a glycosylation shift at the C-26 signal (+7.5 ppm), demonstrating a *β*-glucopyranosyl group to be located at the C-26-OH. Therefore, **1** was formulated as hosenkol B 3-*O*-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranosido-26-*O*-*β*-D-glucopyranoside.

Hosenkoside I (**4**), was obtained as colorless needles. The negative FAB-MS of **4** showed ion peaks at *m/z* 815 $[M-H]^-$ and 653 $[M-C_6H_{10}O_5-H]^-$, suggesting the molecular formula $C_{42}H_{72}O_{15}$. Acid hydrolysis of **4** provided D-glucose and **7**. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **4** indicated the presence of two *β*-D-glucopyranosyl units [H-1′: *δ* 5.15 (d, *J*=7.8 Hz), C-1′: *δ* 105.8; H-1‴: *δ* 4.89 (d, *J*=7.8 Hz), C-1‴: *δ* 105.0]. A crude cellulase treatment of **4** gave **7** and **8**. Comparison of the ^{13}C-NMR spectrum of **4** with that of **8** showed a glycosylation shift for the C-26 signal (+7.6 ppm), demonstrating that a *β*-glucopyranosyl group is located at the C-26-OH. Therefore, **4** was formulated as hosenkol B 3-*O*-*β*-D-glucopyranosido-26-*O*-*β*-D-glucopyranoside.

Hosenkoside G (**2**), $[\alpha]_D^{20}$ +4.0° (pyridine) had the same molecular formula $C_{47}H_{80}O_{19}$ (FAB-MS *m/z* 947 $[M-H]^-$) as **1**. On acid hydrolysis, **2** afforded **7** and hosenkol A (**9**), besides D-glucose and D-xylose in the ratio 2:1. The ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of two *β*-D-glucopyranosyl units [H-1′: *δ* 5.69 (d, *J*=7.8 Hz), C-1′: *δ* 104.2; H-1⁗: *δ* 5.22 (d, *J*=7.3 Hz), C-1⁗: *δ* 105.3], and one *β*-D-xylopyranosyl unit [H-1″: *δ* 5.25 (d, *J*=6.8 Hz), C-1″: *δ* 107.2]. A crude cellulase treatment of **2** gave presapogenin II (**10**), presapogenin III (**11**) and hosenkol C (**12**).[3)]

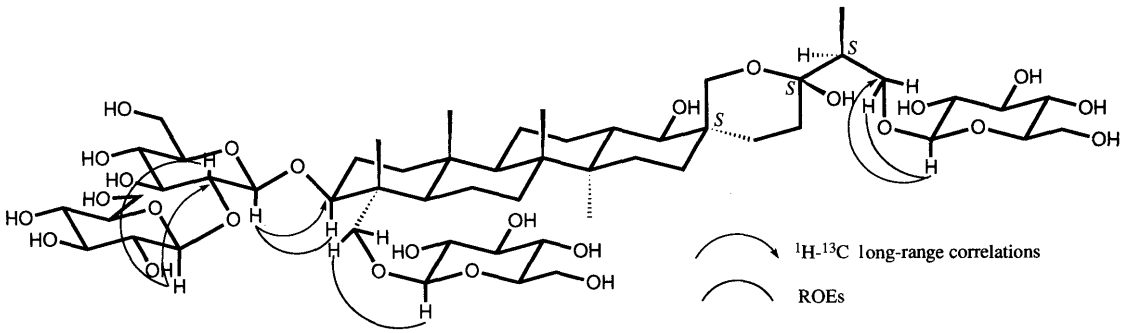
Presapogenin III (**11**), $[\alpha]_D^{20}$ +28.1° (pyridine) revealed a quasi-molecular ion peak at *m/z* 653 $[M-H]^-$ in the negative FAB-MS, suggesting that **11** was a monoglucoside. The C-3 signal in the ^{13}C-NMR spectrum of **11** appeared at lower field by 8.9 ppm than that of **12** because of the glycosylation shift, demonstrating that a *β*-glucopyranosyl group is located at the C-3-OH of the aglycon. Therefore, **11** was determined to be hosenkol C 3-*O*-*β*-D-glucopyranoside.

Presapogenin II (**10**) obtained as colorless needles, had the same molecular formula $C_{42}H_{72}O_{15}$ [FAB-MS, *m/z* 815] as **4**. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **10** indicated the presence of two *β*-D-glucopyranosyl units [H-1′: *δ* 5.56 (d, *J*=7.0 Hz), C-1′: *δ* 106.1; H-1⁗: *δ* 5.30 (d, *J*=7.0 Hz), C-1⁗: *δ* 105.4]. A ^{13}C-NMR spectral comparison of **10** with **11** showed a glycosylation shift at the C-28 signal (+7.1 ppm), demonstrating a *β*-glucopyranosyl group to be located at the C-28-OH. Therefore, **10** was formulated as hosenkol C 3-*O*-*β*-D-glucopyranosido-28-*O*-*β*-D-glucopyranoside. The unsettled sugar sequence of **2** was determined by the combination of ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra with an HMBC experiment. The HMBC spectrum of **2** showed long-range correlations between H-1′ (*δ* 5.69) of the glucose and C-3 (*δ* 81.6), H-1″ (*δ* 5.25) of the xylose and C-2′ (*δ* 84.6) of glucose, and H-1⁗ (*δ* 5.22) of the glucose and C-28 (*δ* 71.5), indicating a *β*-sambubiosyl (Xyl^{2}Glc) unit to be located at C-3-OH and a *β*-glucosyl unit at C-28-OH. Hence, **2** was formulated as hosenkol C 3-*O*-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranosido-28-*O*-*β*-D-glucopyranoside.

Hosenkoside J (**5**), $[\alpha]_D^{20}$ +23.2° (MeOH), had the molecular formula $C_{42}H_{72}O_{15}\cdot 7/2H_2O$ based on the elementary analysis. On acid hydrolysis, **5** afforded D-glucose, besides **9**. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **5** indicated the presence of two *β*-D-glucopyranosyl units [H-1″: *δ* 5.37 (d, *J*=7.3 Hz), C-1″: *δ* 105.9; H-1′: *δ* 5.10 (d, *J*=7.3 Hz), C-1′: *δ* 103.9]. A crude cellulase treatment of **5** gave **9** and presapogenin IV (**13**).

Presapogenin IV (**13**), $[\alpha]_D^{20}$ +37.9° (pyridine) revealed a quasi-molecular ion peak at *m/z* 653 $[M-H]^-$ in the negative FAB-MS, suggesting that **13** was a monoglucoside. Comparison of the ^{13}C-NMR spectrum of **13** with that of **9** showed that the signals of the C-2 and C-3 in **13** were shifted by −1.7 ppm and +8.7 ppm, respectively, indicating that a *β*-glucopyranosyl group is joined to the C-3-OH. Therefore, **13** was formulated as hosenkol A 3-*O*-*β*-D-glucopyranoside. Comparison of the ^{13}C-NMR spectrum of **5** with that of **13** showed a glycosylation shift for the C-2 signal (+8.6 ppm) of glucosyl, demonstrating that a *β*-glucopyranosyl group is located at the C-2-OH of glucose. Therefore, **5** was formulated as hosenkol A 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside.

Hosenkoside K (**6**), one of the major saponins, $[\alpha]_D^{20}$ +16.3° (MeOH), had the molecular formula $C_{54}H_{92}O_{25}\cdot 13/2H_2O$ based on the elementary analysis. On acid hydrolysis, **6** afforded **9** and D-glucose. The ^{1}H-, ^{13}C- and ^{1}H–^{13}C COSY spectra of **6** indicated the presence of four *β*-D-glucopyranosyl units [H-1′: *δ* 5.69 (d, *J*=7.3 Hz), C-1′: *δ* 104.6; H-1″: *δ* 5.33 (d, *J*=7.3 Hz), C-1″: *δ* 106.2; H-1⁗: *δ* 5.28 (d, *J*=7.5 Hz), C-1⁗: *δ* 105.4; H-1‴: *δ* 4.84 (d, *J*=7.5 Hz), C-1‴: *δ* 105.3]. A crude cellulase treatment of **6** gave **9**, **13**, and hosenkosides A (**14**) and D (**15**).[3)] The remaining sugar sequence of **6** was determined as follows. The C-26 signal in the ^{13}C-NMR spectrum of **6** appeared at lower field by 7.7 ppm than that of **14** because of the glycosylation shift, demonstrating that a *β*-glucopyranosyl group is located at the C-26-OH of the aglycon. In the HMBC spectrum of **6**, long-range correlations were seen between H-1′ (*δ* 5.69) of the glucose and C-3 (*δ* 81.8), H-1″ (*δ* 5.33) of the glucose and C-2′ (*δ* 83.9) of glucose, and H-1‴ (*δ* 4.84) of the glucose and C-26 (*δ* 72.1), indicating a *β*-sophorosyl unit to be located at C-3-OH and a



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β-glucosyl unit to be located at C-26-OH. Further, the nuclear Overhauser effect (NOE) was also observed between C-3-H (*ca.* δ 4.44) and H-1′ (δ 5.69) of the glucose, H-2′ (*ca.* δ 4.28) of the glucose and H-1″ (δ 5.33) of the glucose, C-28-H (δ 4.54) and H-1‴ (δ 5.28) of the glucose, and C-26-H (*ca.* δ 4.23) and H-1‴ (δ 4.84) in the ROESY experiment. Hence, **6** was formulated as hosenkol A 3-*O*-β-D-glucopyranosyl(1→2)-*O*-β-D-glucopyranosido-26-*O*-β-D-glucopyranosido-28-*O*-β-D-glucopyranoside. As far as we know, hosenkoside K is the first example of a tridesmosidic baccharane saponin.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus without correction. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a JEOL GX-400 or Varian UNITY 600 spectrometer in C_5D_5N solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H-COSY, heteronuclear multiple quantum coherence (HMQC), insensitive nuclei enhanced by polarization transfer (INEPT), TOCSY, ROESY and HMBC (512 × 1024 data matrix size, 128 scans, recycle delay = 1.16 s). Coupling constants (*J* values) are given in hertz (Hz). The HR-EI-MS and FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as the matrix) were measured on JEOL JMS-HX-100 and JEOL JMS-PX303 mass spectrometers, respectively. For column chromatography, Silica gel 60 (40—63 μm, Merck) and Silica gel 60 silanized (63—200 μm, Merck) were used. TLC was carried out on Silica gel 60F-254 (Merck) with $CHCl_3$–MeOH–H_2O (65 : 30 : 4), and Silica gel 60 silanized with MeOH–H_2O (1 : 1). For enzymatic hydrolysis, cellulase from *Aspergillus niger* (Sigma, type I) was used.

Isolation of Saponins The seeds were purchased from Sakata Seed Co., Yokohama, Japan. Powdered dried seeds (2.0 kg) of *Impatiens balsamina* were defatted with *n*-hexane and extracted with 50% MeOH at 60 °C. The methanolic extract (150 g) was partitioned between H_2O and EtOAc. The water layer further partitioned between H_2O and *n*-BuOH. The butanolic layer (60 g) was chromatographed on a silica gel column with EtOAc–MeOH (4 : 1) to give saponin fractions. These saponin factions (15 g) were repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (65 : 30 : 4) to give five fractions, frs. I—V in order of elution. Fraction III was repeatedly column chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (25 : 6 : 1) and on silanized silica gel with 50% MeOH to give hosenkosides D (**15**) E, F (**1**, 3.0 g), G (**2**, 1.75 g), H (**3**, 1.0 g), I (**4**, 0.35 g), and J (**5**, 0.25 g). Fraction IV was repeatedly column chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (25 : 8 : 1) and on silanized silica gel with 50% MeOH to give hosenkosides A (**14**), B, C, K (**6**, 0.32 g).

Hosenkoside F (**1**): mp 230—232 °C (MeOH), $[\alpha]_D^{20}$ + 7.2° (*c* = 0.94, pyridine). FAB-MS *m/z* 947 $[M-H]^-$. *Anal.* Calcd. for $C_{47}H_{80}O_{19}\cdot H_2O$: C, 58.37; H, 8.55. Found: C, 58.10; H, 8.60. ^{1}H-NMR δ: 0.87, 0.92, 0.98, 1.06 (3H each, s, *tert*-CH_3), 1.10 (3H, d, *J* = 6.8 Hz, H-27), 2.18 (1H, m, H-25), 3.33 (1H, d, *J* = 10.3 Hz, H-17), 4.00, 4.30 (each 1H, d, *J* = 10.5 Hz, H-21), *ca.* 4.40 (1H, m, H-3), 4.87 (1H, d, *J* = 7.8 Hz, H-1‴ of Glc), 5.15 (1H, d, *J* = 6.8 Hz, H-1′ of Glc), 5.24 (1H, d, *J* = 6.4 Hz, H-1′ of Xyl). For ^{13}C-NMR data, see Table I.

Hosenkoside G (**2**): mp 223—225 °C (MeOH), $[\alpha]_D^{20}$ + 4.0° (*c* = 1.3, pyridine). FAB-MS *m/z* 947 $[M-H]^-$. *Anal.* Calcd for $C_{47}H_{80}O_{19}\cdot H_2O$: C, 58.37; H, 8.55. Found: C, 58.18; H, 8.57. ^{1}H-NMR δ: 0.82, 0.85, 0.91, 1.04, 2.03 (3H each, s, *tert*-CH_3), 3.64 (1H, d, *J* = 10.5 Hz, H-17), 3.80, 4.50 (each 1H, d, *J* = 11.0 Hz, H-21), *ca.* 4.45 (1H, m, H-3), 4.51, 4.53 (each 1H, d, *J* = 11.0 Hz, H-26), 5.22 (1H, d, *J* = 7.3 Hz, H-1⁗ of Glc), 5.25 (1H, d, *J* = 6.8 Hz, H-1″ of Xyl), 5.49 (1H, t, *J* = 7.0 Hz, H-24), 5.69 (1H, d, *J* = 7.8 Hz, H-1′ of Glc). For ^{13}C-NMR data, see Table I.

Hosenkoside H (**3**): An amorphous powder, $[\alpha]_D^{20}$ + 15.1° (*c* = 1.2, MeOH). FAB-MS *m/z* 785 $[M-H]^-$. *Anal.* Calcd for $C_{41}H_{70}O_{14}\cdot H_2O$: C, 64.26; H, 9.59. Found: 64.52; H, 9.63. ^{1}H-NMR δ: 0.88, 0.92, 0.98, 1.06 (3H each, s, *tert*-CH_3), 1.15 (3H, d, *J* = 6.8 Hz, H-27), 2.10 (1H, m, H-25), 3.72, 4.20 (each 1H, d, *J* = 11.0 Hz, H-28), 3.33 (1H, d, *J* = 10.3 Hz, H-17), 4.10, 4.30 (each 1H, d, *J* = 11.0 Hz, H-21), *ca.* 4.40 (1H, m, H-3), 5.15 (1H, d, *J* = 7.8 Hz, H-1′ of Glc), 5.24 (1H, d, *J* = 6.8 Hz, H-1″ of Xyl). For ^{13}C-NMR data, see Table I.

Hosenkoside I (**4**): mp 278-280°C (MeOH), $[\alpha]_D^{20}$ + 12.0° (*c* = 1.7, MeOH). FAB-MS *m/z* 815 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot H_2O$: C, 57.81; H, 8.49. Found: C, 57.92; H, 8.78. ^{1}H-NMR δ: 0.86, 0.93, 0.98, 0.98 (3H each, s, *tert*-CH_3), 1.10 (3H, d, *J* = 6.8 Hz, H-27), 2.20 (1H, m, H-25), 3.33 (1H, d, *J* = 10.3 Hz, H-17), 3.73, 4.20 (each 1H, d, *J* = 11.0 Hz, H-28), 4.01, 4.30 (each 1H, d, *J* = 10.5 Hz, H-21), *ca.* 4.40 (1H, m, H-3), 4.89 (1H, d, *J* = 7.8 Hz, H-1‴ of 26-Glc), 5.15 (1H, d, *J* = 7.8 Hz, H-1′ of 3-Glc). For ^{13}C-NMR data, see Table I.

Hosenkoside J (**5**): mp 241—243 °C (MeOH), $[\alpha]_D^{20}$ + 23.2° (*c* = 5.3, MeOH). FAB-MS *m/z* 815 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot 7/2H_2O$: C, 57.32; H, 9.05. Found: C, 57.46; H, 8.65. ^{1}H-NMR δ: 0.84, 0.87, 0.91, 1.08 (3H each, s, *tert*-CH_3), 1.10 (3H, d, *J* = 6.6 Hz, H-27), 2.13 (1H, m, H-25), 3.28, 4.57 (each 1H, d, *J* = 11.7 Hz, H-21), 3.42 (1H, d, *J* = 10.2 Hz, H-17), *ca.* 4.40 (1H, m, H-3), 5.10 (1H, d, *J* = 7.3 Hz, H-1′ of Glc), 5.37 (1H, d, *J* = 7.3 Hz, H-1″ of Glc). For ^{13}C-NMR data, see Table I.

Hosenkoside K (**6**): mp 160—162 °C (MeOH), $[\alpha]_D^{20}$ + 16.3° (*c* = 7.0, MeOH). FAB-MS *m/z* 1139 $[M-H]^-$, 977 $[M-C_6H_{10}O_5-H]^-$. *Anal.* Calcd for $C_{54}H_{92}O_{25}\cdot 13/2H_2O$: C, 51.54; H, 8.41. Found: C, 51.47; H, 8.04. ^{1}H-NMR δ: 0.75, 0.80, 0.85, 1.03 (3H each, s, *tert*-CH_3), 1.02 (3H, d, *J* = 6.6 Hz, H-27), 2.17 (1H, m, H-25), 3.39 (1H, d, *J* = 10.7 Hz, H-17), 3.25, 4.54 (each 1H, d, *J* = 11.9 Hz, H-21), 4.23, 4.54 (each 1H, d, *J* = 10.5 Hz, H-28), *ca.* 4.44 (1H, m, H-3), 4.84 (1H, d, *J* = 7.5 Hz, H-1‴ of Glc), 5.28 (1H, d, *J* = 7.5 Hz, H-1⁗ of Glc), 5.33 (1H, d, *J* = 7.3 Hz, H-1″ of Glc), 5.69 (1H, d, *J* = 7.3 Hz, H-1′ of Glc). For ^{13}C-NMR data, see Table I.

Acid Hydrolysis of Hosenkoside H (3) A solution of **3** (30 mg) in 5% H_2SO_4–EtOH (1 : 95, 10 ml) was heated at 100 °C for 3 h. The reaction mixture was extracted with ether. The organic layer was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (25 : 8 : 0.1) to give hosenkol B (**7**, 15 mg). Compound **7**, mp 265—267 °C (MeOH), $[\alpha]_D^{20}$ + 50.4° (*c* = 1.14, pyridine). HR-EI-MS obsd. for $[M(C_{30}H_{52}O_5)-H_2O]$ 474.3705, Calcd 474.3709. ^{1}H-NMR δ: 0.92, 0.92, 1.00, 1.06 (3H each, s, *tert*-CH_3), 1.15 (3H, d, *J* = 7.1 Hz, H-27), 2.10 (1H, m, H-25), 3.32 (1H, d, *J* = 10.7 Hz, H-17), 3.48 (1H, m, H-24), 3.71, 4.18 (each 1H, d, *J* = 11.0 Hz, H-28), 3.89 (1H, dd, *J* = 10.2, 5.3 Hz, H-26), 4.08 (1H, dd, *J* = 10.2, 5.0 Hz, H-26), 4.07, 4.39 (each 1H, d, *J* = 10.5 Hz, H-21), 4.20 (1H, dd, *J* = 12.0, 5.0 Hz, H-3). For ^{13}C-NMR data, see Table I. The aqueous layer was neutralized with Amberlite IR-45 and evaporated *in vacuo* to dryness. The sugar was determined by using refractive index (RI) detection (Waters 410) and chiral detection (Shodex OR-1), respectively, in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min,

TABLE I. ^{13}C-NMR Data for Compounds **1**—**15** (Pyridine-d_5, δ-Values)

C No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	39.2	38.8	39.2	39.1	39.0	38.8	39.1	39.1	38.8	39.2	39.1	39.1	39.1	38.8	38.8
2	26.6	26.6	26.4	26.1	25.9	26.6	27.8	26.1	26.4	26.2	27.9	27.8	26.1	26.6	26.4
3	81.7	81.6	81.7	82.0	82.5	81.8	73.3	81.9	81.7	82.2	73.3	73.3	82.0	81.9	81.8
4	43.7	43.3	43.7	43.5	43.5	43.3	43.0	43.6	43.2	43.7	43.0	43.0	43.6	43.3	43.2
5	47.6	47.8	47.6	47.8	48.3	47.8	48.8	47.8	47.8	48.0	48.8	48.8	48.0	47.8	47.8
6	18.1	17.9	18.1	18.1	18.1	17.9	18.5	18.1	17.9	18.3	18.5	18.5	18.2	17.9	17.8
7	33.7	33.7	33.7	33.6	33.5	33.7	33.6	33.6	33.6	33.8	33.6	33.6	33.6	33.7	33.6
8	41.0	40.9	41.0	41.0	40.8	40.8	41.0	41.0	40.9	41.1	41.0	40.9	40.8	40.8	40.8
9	51.3	51.0	51.3	51.2	51.2	51.0	51.2	51.2	51.0	51.4	51.2	51.3	51.1	51.1	51.0
10	36.9	36.9	36.9	37.0	36.9	36.9	37.3	37.0	36.9	37.2	37.3	37.3	36.9	36.9	36.9
11	21.2	21.2	21.2	21.2	21.2	21.2	21.2	21.2	21.2	21.2	21.3	21.3	21.2	21.2	21.2
12	25.7	25.3	25.6	25.6	24.8	24.9	25.6	25.6	25.3	25.5	25.3	24.8	24.9	24.9	24.9
13	39.9	40.6	39.9	39.9	41.7	41.7	39.9	39.9	40.6	40.6	40.5	41.7	41.8	41.8	41.8
14	42.2	42.5	42.2	42.2	42.2	42.2	42.2	42.2	42.5	42.7	42.5	42.2	42.2	42.2	42.1
15	26.5	26.7	26.4	26.4	26.7	26.7	26.4	26.4	26.7	26.9	26.7	26.7	26.7	26.8	26.7
16	26.5	28.2	26.6	26.5	32.9	32.7	26.6	26.6	28.2	28.4	28.3	32.9	32.9	32.9	32.9
17	76.1	77.4	76.1	76.1	79.8	79.9	76.0	76.1	77.3	77.4	77.3	79.8	79.8	79.9	79.8
18	15.9	15.7	15.9	15.9	15.7	15.7	15.9	15.9	15.7	16.0	15.9	15.8	15.6	15.7	15.6
19	17.1	17.3	17.1	17.1	17.1	17.4	16.9	17.1	17.3	17.3	17.0	17.0	17.4	17.4	17.3
20	38.3	41.5	38.4	38.3	36.0	36.0	38.4	38.4	41.5	41.7	41.5	35.9	35.9	36.0	35.9
21	67.8	65.0	67.8	67.7	72.3	72.2	67.8	67.8	65.0	64.7	65.0	72.4	72.6	72.6	72.5
22	34.4	38.0	34.5	34.4	37.4	37.3	34.5	34.5	38.0	38.1	38.0	37.6	37.4	37.7	37.6
23	24.5	22.0	24.9	24.5	26.1	25.7	24.9	24.9	22.0	22.0	21.9	26.0	26.0	26.1	26.0
24	79.7	127.9	80.7	79.7	79.5	78.9	80.6	80.6	127.9	128.1	127.8	79.6	79.7	79.7	79.7
25	39.5	136.0	41.9	39.5	40.4	38.1	41.9	41.9	136.0	136.1	136.0	40.5	40.5	40.6	40.6
26	72.5	60.9	65.0	72.5	64.3	72.1	65.0	64.9	60.9	61.0	60.8	64.4	64.3	64.4	64.4
27	13.7	21.9	13.6	13.7	13.5	13.9	13.5	13.6	21.9	22.0	21.8	13.4	13.5	13.5	13.5
28	63.5	71.5	63.5	64.5	64.8	72.1	67.8	64.5	71.8	64.7	67.8	67.8	64.5	71.8	71.8
29	13.0	12.8	13.0	13.4	13.2	13.3	12.9	13.4	13.4	13.6	12.9	12.9	13.4	13.4	13.3
30	15.3	15.2	15.2	15.2	14.9	15.1	15.2	15.2	15.1	15.2	15.0	14.9	15.0	15.1	15.0
3-*O*-Gly (Glc^{2}Xyl or Glc^{2}Glc or Glc)															
1′	104.2	104.2	104.2	105.8	103.9	104.6		105.8		106.1	105.9		105.9	104.6	105.8
2′	84.5	84.6	84.5	75.8	83.9	83.9		75.8		75.9	76.0		75.3	83.8	75.3
3′	78.3	78.3	78.3	78.6	78.0	78.1		78.6		78.6	78.8		78.5	78.2	78.6
4′	71.5	71.9	71.5	71.7	71.2[a]	71.9		71.7		71.8	71.8		71.7	71.9	71.8
5′	77.9	78.0	77.9	78.3	78.3	78.0		78.3		78.3	78.4		78.2	78.1	78.3
6′	62.8	63.0	62.8	62.9	62.6	63.0		62.9		63.0	63.0		63.0	63.0	63.0
1″	107.1	107.2	107.1		105.9	106.2								106.1	
2″	76.5	76.5	76.5		76.8	77.3								77.1	
3″	78.2	78.2	78.2		78.3	78.5								78.5	
4″	71.0	71.0	71.0		71.3[a]	71.7								71.7	
5″	67.6	67.5	67.5		78.0	77.7								77.7	
6″					62.5	62.8								62.8	
26-*O*-Glc															
1‴	105.0			105.0		105.3									
2‴	75.2			75.2		75.4									
3‴	78.6			78.6		78.6									
4‴	71.7			71.7		71.6									
5‴	78.4			78.4		78.5									
6‴	62.8			62.8		62.8									
28-*O*-Glc															
1⁗		105.3				105.4				105.4				105.3	106.1
2⁗		75.5				75.5				75.3				75.5	75.9
3⁗		78.5				78.5				78.7				78.5	78.7
4⁗		71.9				72.0				72.0				71.9	72.0
5⁗		77.6				78.0				77.9				77.9	77.9
6⁗		63.0				63.1				63.2				63.1	63.2

a) Assignments may be interchanged.

70 °C) by comparison with authentic sugars (10 mM each of D-Glc and D-Xyl). The sugar part gave positive peaks at 5.75 min (D-Xyl, 5.73 min), and 7.38 min (D-Glc, 7.36 min).

Enzymatic Hydrolysis of Hosenkoside H (3) A solution of **3** (200 mg) in EtOH (5 ml) and 0.01 M NaH_2PO_4 buffer (pH 4.0, 45 ml) was incubated with crude cellulase (200 mg, Sigma) for 2 d at 37 °C and worked up as usual. The crude product was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25 : 8 : 0.1) giving **3** (15 mg), hosenkol B (**7**, 20 mg) and presapogenin I (**8**, 15 mg). Compound **8**, mp 232—234 °C (MeOH), $[\alpha]_D^{20}$ + 30.2° (c = 1.26, pyridine). FAB-MS m/z 653 $[M-H]^-$. *Anal.* Calcd for $C_{36}H_{62}O_{10}$: C, 66.02; H, 9.54. Found: C, 65.70; H, 9.69. ^{1}H-NMR δ: 0.85, 0.92, 1.00, 1.00 (3H each, s, *tert*-CH_3), 1.16 (3H, d, J = 6.8 Hz, H-27), 2.12 (1H, m, H-25), 3.34 (1H, d, J = 10.3 Hz, H-17), 3.74, 4.24 (each 1H, d, J = 11.0 Hz, H-28), 4.10, 4.32 (each 1H, d, J = 11.0 Hz, H-21), *ca.* 4.44 (1H, m, H-3), 5.17 (1H, d, J = 7.0 Hz, H-1′ of Glc).

Acid Hydrolysis of Hosenkoside F (1) Acid hydrolysis of **1** (10 mg) was carried out in the same way as described for **3** to give **7** (5 mg) as

well as D-Glc and D-Xyl.

Enzymatic Hydrolysis of Hosenkoside F (1) Enzymatic hydrolysis of **1** (100 mg) was carried out in the same way as described for **3** to give **3** (20 mg), **7** (10 mg) and **8** (5 mg).

Acid Hydrolysis of Hosenkoside I (4) Acid hydrolysis of **4** (10 mg) was carried out in the same way as described for **3** to give **7** (5 mg) as well as D-Glc.

Enzymatic Hydrolysis of Hosenkoside I (4) Enzymatic hydrolysis of **4** (40 mg) was carried out in the same way as described for **3** to give **7** (10 mg) and **8** (5 mg).

Acid Hydrolysis of Hosenkoside G (2) Acid hydrolysis of **2** (20 mg) was carried out in the same way as described for **3** to give **7** (5 mg) and **9** (4 mg) as well as D-Glc and D-Xyl.

Enzymatic Hydrolysis of Hosenkoside G (2) Enzymatic hydrolysis of **2** (200 mg) was carried out in the same way as described for **3** to give presapogenin II (**10**, 40 mg), presapogenin III (**11**, 25 mg) and hosenkol C (**12**, 15 mg). Compound **10**, mp 199—201 °C (MeOH), $[\alpha]_D^{20}$ + 11.5° (c=0.66, pyridine). FAB-MS m/z 815 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot 3/2H_2O$: C, C, 59.77; H, 8.96. Found: C, 59.90; H, 8.80. ^{1}H-NMR δ: 0.80, 0.85, 0.90, 0.90, 2.02 (3H each, s, *tert*-CH_3), 3.64 (1H, d, J=11.0 Hz, H-17), *ca.* 4.40 (1H, m, H-3), *ca.* 4.52 (2H, m, H-26), 5.30 (1H, d, J=7.0 Hz, H-1'''' of Glc), 5.50 (1H, t, J=6.5 Hz, H-24), 5.56 (1H, d, J=7.0 Hz, H-1' of Glc). For ^{13}C-NMR data, see Table I. Compound **11**, mp 225—227 °C (MeOH), $[\alpha]_D^{20}$ + 28.1° (c=1.14, pyridine). FAB-MS m/z 653 $[M-H]^-$. *Anal.* Calcd for $C_{36}H_{62}O_{10}$: C, 66.02; H, 9.54. Found: C, 65.80; H, 9.60. ^{1}H-NMR δ: 0.80, 0.85, 0.90, 0.90, 2.02 (3H each, s, *tert*-CH_3), 3.63 (1H, d, J= 11.5 Hz, H-17), *ca.* 4.40 (1H, m, H-3), *ca.* 4.52 (2H, m, H-26), 5.17 (1H, d, J=8.0 Hz, H-1' of Glc), 5.50 (1H, t, J=6.5 Hz, H-24). For ^{13}C-NMR data, see Table I. Compound **12**, mp 173—175 °C (MeOH), $[\alpha]_D^{20}$ + 43.3° (c= 0.6, pyridine). HR-EI-MS obsd. for $[M(C_{30}H_{52}O_5)-H_2O]$ 474.3705, Calcd 474.3709. ^{1}H-NMR δ: 0.92, 0.94, 1.01, 1.07, 2.02 (3H each, s, *tert*-CH_3), 3.63 (1H, d, J= 10.5 Hz, H-17), 3.72, 4.18 (each 1H, d, J=10.2 Hz, H-28), 3.81, 4.56 (each 1H, d, J=10.5 Hz, H-21), 4.22 (1H, dd, J=10.0, 6.0 Hz, H-3), 4.52, 4.56 (each 1H, d, J=11.0 Hz, H-26), 5.48 (1H, t, J=6.8 Hz, H-24). For ^{13}C-NMR data, see Table I.

Acid Hydrolysis of Hosenkoside J (5) Acid hydrolysis of **5** (10 mg) was carried out in the same way as described for **3** to give **9** (5 mg) as well as D-Glc. Compound **9**, mp 225—227 °C (MeOH), $[\alpha]_D^{20}$ + 78.9° (c=1.51, pyridine). HR-EI-MS obsd. for $C_{30}H_{52}O_5$ 492.3796, Calcd 492.3814. ^{1}H-NMR δ: 0.84, 0.95, 0.96, 1.06 (3H each, s, *tert*-CH_3), 1.00 (3H, d, J=6.8 Hz, H-27), 2.13 (1H, m, H-25), 3.43 (1H, d, J=10.0 Hz, H-17), 3.54 (1H, m, H-24), 3.28, 4.59 (each 1H, d, J=12.0 Hz, H-21), 3.72, 4.19 (each 1H, d, J=11.0 Hz, H-28), 3.87 (1H, dd, J=10.5, 6.0 Hz, H-26), 4.01 (1H, dd, J=10.5, 5.0 Hz, H-26), 4.22 (1H, dd, J=12.0, 5.0 Hz, H-3). For ^{13}C-NMR data, see Table I.

Enzymatic Hydrolysis of Hosenkoside J (5) Enzymatic hydrolysis of **5** (50 mg) was carried out in the same way as described for **3** to give **9** (10 mg) and presapogenin IV (**13**, 15 mg). Compound **13**, mp 285—287 °C (MeOH), $[\alpha]_D^{20}$ + 37.9° (c=0.7, pyridine). FAB-MS m/z 653 $[M-H]^-$. *Anal.* Calcd for $C_{36}H_{62}O_{10}\cdot H_2O$: C, 64.26; H, 9.59. Found: C, 64.52; H, 9.63. ^{1}H-NMR δ: 0.76, 0.80, 0.85, 0.89 (3H each, s, *tert*-CH_3), 1.10 (3H, d, J=6.5 Hz, H-27), 2.13 (1H, m, H-25), 3.26, 4.57 (each 1H, d, J=11.5 Hz, H-21), 3.42 (1H, d, J=10.2 Hz, H-17), 3.74, 4.25 (each 1H, d, J=11.0 Hz, H-28), *ca.* 4.40 (1H, m, H-3), 5.17 (1H, d, J=7.0 Hz, H-1' of Glc). For ^{13}C-NMR data, see Table I.

Enzymatic Hydrolysis of Hosenkoside K (6) Enzymatic hydrolysis of **6** (100 mg) was carried out in the same way as described for **3** to give **9** (10 mg), **13** (10 mg), **14** (10 mg) and **15** (5 mg). Compound **14**, mp 234—236 °C (MeOH), $[\alpha]_D^{20}$ + 20.9° (c=1.3, pyridine). FD-MS m/z 1001 $[M+Na]^+$. *Anal.* Calcd for $C_{48}H_{82}O_{20}\cdot H_2O$: C, 57.81; H, 8.49. Found: C, 57.92; H, 8.78. ^{1}H-NMR δ: 0.75, 0.79, 0.85, 1.05 (3H each, s, *tert*-CH_3), 1.09 (3H, d, J=6.8 Hz, H-27), 2.13 (1H, m, H-25), 3.28, 4.55 (each 1H, d, J=11.7 Hz, H-21), 3.45 (1H, d, J=10.2 Hz, H-17), *ca.* 4.40 (1H, m, H-3), 5.28 (1H, d, J=7.2 Hz, H-1'''' of 28-Glc), 5.36 (1H, d, J=7.2 Hz, H-1'' of Glc), 5.68 (1H, d, J=8.2 Hz, H-1' of Glc). For ^{13}C-NMR data, see Table I. Compound **15**, mp 241—243 °C (MeOH), $[\alpha]_D^{20}$ + 16.9° (c=1.1, pyridine). FAB-MS m/z 815 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot 5/2H_{2O}$: C, 58.52; H, 9.00. Found: C, 58.75; H, 8.66. ^{1}H-NMR δ: 0.76, 0.80, 0.85, 0.89 (3H each, s, *tert*-CH_3), 1.10 (3H, d, J=6.8 Hz, H-27), 2.12 (1H, m, H-25), 3.25, 4.58 (each 1H, d, J=11.7 Hz, H-21), 3.42 (1H, d, J=10.2 Hz, H-17), *ca.* 4.40 (1H, m, H-3), 5.29 (1H, d, J=7.8 Hz, H-1'''' of Glc), 5.56 (1H, d, J=7.8 Hz, H-1' of 3-Glc). For ^{13}C-NMR data, see Table I.

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