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Hosenkosides A, B, C, D, and E, Novel Baccharane Glycosides from the Seeds of *Impatiens balsamina*

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Abstract: From the seeds of *Impatiens balsamina* has been isolated five novel baccharane glycosides, hosenkosides A-E. The structures of all isolates were secured by the use of 2D NMR techniques (^{1}H-^{1}H COSY, HMQC, HMBC, ROESY, TOCSY), CD spectroscopy and chemical derivatization.

INTRODUCTION

Impatiens balsamina L., an annual native of India, is now widely cultivated as an ornamental plant. The seeds have been used to treat difficult labour, 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 of one of the major saponins, hosenkoside A (**1**) [3] from the seeds of *I. balsamina* and the relative structure of hosenkol A, the genin of hosenkoside A, being (3*S*,4*R*, 17*R*,20*S*,24*S*,25*S*)-3,17,26,28-tetrahydroxy-21,24-epoxybaccharane (**6**) or its isomer, on the basis of X-ray analysis. In this paper, we reported the full structure of hosenkoside A, and the isolation and structural elucidation of four additional novel saponins hosenkosides B-E (**2**-**5**), having a rare baccharane skeleton. Their structure were elucidated by chemical and spectral methods, 2D-NMR techniques and CD spectroscopy being practically helpful.

RESULTS AND DISCUSSION

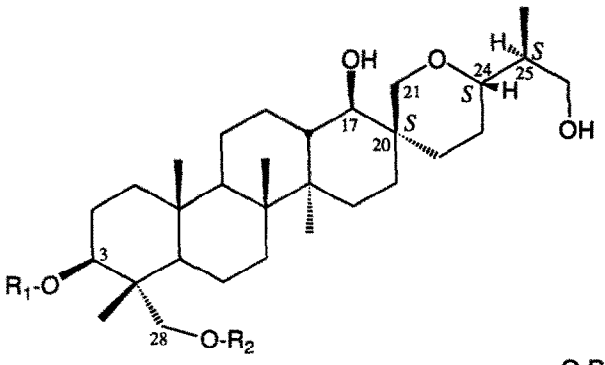
The 50% MeOH extract of the seed of *Impatiens balsamina*, followed by treatment with *n*-BuOH gave a saponin fraction. Repeated separation of the saponin fraction by ordinary-phase SiO_2 and reversed-phase (silanised SiO_2) furnished four new saponins hosenkosides B (**2**), C (**3**), D (**4**), and E (**5**), besides hosenkoside A (**1**). A cellulase treatment of hosenkosides A (**1**) and D (**4**) gave hosenkol A (**6**) for aglycon, mp 225-227 °C, $[\alpha]_D^{20}$ + 78.6° (py), $C_{30}H_{52}O_5$ showed a molecular ion peak at *m/z* 492.3796 in the high-resolution mass

measurement (HRMS). The relative structure of **6** was determined by analysis of NMR data including ^{1}H-^{1}H COSY, HMQC, HMBC and ROESY experiments (partial structure **A**), and final by X-ray analysis. Thus, **6** is shown to be (3*S*,4*R*,17*R*,20*S*,24*S*,25*S*)-3,17,26,28-tetrahydroxy-21,24-epoxybaccharane or its isomer. Determination of the absolute configuration of **6** was achieved using the empirical cd exciton chirality method employing the stronger chelating reagent Eu(fod)$_3$.[4,5] Acetonide (**6a**) of **6** was acetylated with acetic anhydride in pyridine, followed by removing the protection group with acid to give hosenkol A 17,26-diacetate (**6b**). Compound **6b** in the presence of Eu(fod)$_3$ showed a positively split cotton effect, $\Delta\varepsilon_{310}$+0.37, referring to the data of methyl hederagenin[6] indicating the C-3 *S* configuration. Hence, hosenkol A was characterized as (3*S*,4*R*,17*R*,20*S*,24*S*,25*S*)-3,17,26,28-tetrahydroxy-21,24-epoxybaccharane.

Table 1. ^{1}H-NMR data for compounds **6, 8, 12** (Py-d$_5$, δ-values)

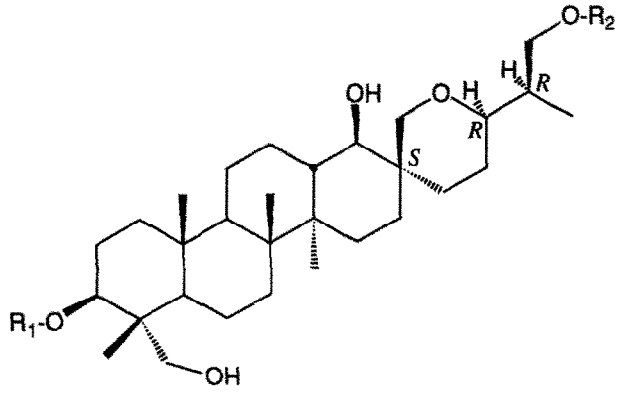
	6	**8**	**12**
H-3	4.22 dd, *J* 12.0, 5.0	4.20 dd, *J* 11.2, 5.3	4.22 dd, *J* 10.0, 6.0
H-13	1.77 ddd, *J* 10.0,10.0,4.0	1.78 ddd, *J* 10.7,10.7,4.0	2.09 ddd, *J* 10.0,10.0,3.5
Hα-16	ca 1.22 m	ca 1.72 m	1.42ddd, *J* 10.0, 10.0,3.0,
Hβ-16	ca 1.22 m	2.32 ddd, *J* 13.0, 3.5, 3.5	ca 1.50 m
H-17	3.43 d, *J* 10.0	3.32 d, *J* 10.7	3.63 d, *J* 10.5
H-18	0.96 s	1.00 s	0.94 s
H-19	0.95 s	0.92 s	1.01 s
H$_A$-21	3.28 d, *J* 12.0 †	4.07 d, *J* 10.5 †	3.81 d, *J* 11.0
H$_B$-21	4.59 dd, *J* 12.0, 1.5 ‡	4.39 dd, *J* 10.5, 1.5 ‡	4.56 d, *J* 11.0
H$_A$-22	1.34 ddd, *J* 12.0, 12.0, 5.0 †	1.36 ddd, *J* 13.2, 4.0, 4.0 †	ca 1.68 m
H$_B$-22	ca1.94 m ‡	2.59 ddd, *J* 13.2, 13.2, 4.0 ‡	2.29 m
H$_A$-23	2.27 dddd, *J* 12.0, 12.0, 12.0, 4.0 †	1.58 dddd, *J* 13.0, 4.0, 4.0, 4.0 †	ca1.98 m
H$_B$-23	ca 1.68 m ‡	ca 1.73 m ‡	ca 2.42 m
24	3.54 ddd, 12.0, 11.0, 3.0	3.48 ddd, 12.0, 11.0, 3.0	5.48 t, *J* 6.8
25	2.13 dddq, *J* 6.0, 6.0, 5.0, 6.8	2.10 m	
H$_A$-26	3.87 dd, *J* 10.5, 6.0	3.89 dd, *J* 10.2, 6.3	4.52 d, *J* 11.0
H$_B$-26	4.01 dd, *J* 10.5, 5.0	4.08 dd, *J* 10.2, 5.0	4.56 d, *J* 11.0
27	1.00 d, *J* 6.8	1.15 d, *J* 7.1	2.02 s
28	3.72 d, *J* 11.0, 4.19 d, *J* 11.0	3.71 d, *J* 11.0, 4.18 d, *J* 11.0	3.72 d, *J* 10.2, 4.18 d, *J*10.2
29	1.06 s	1.06 s	0.92 s
30	0.84 s	0.92 s	1.07 s

† α-Hydrogen (pro-*R*), distinguished from pro-*S* hydrogen by NOE experiments.
‡ β-Hydrogen (pro-*S*).

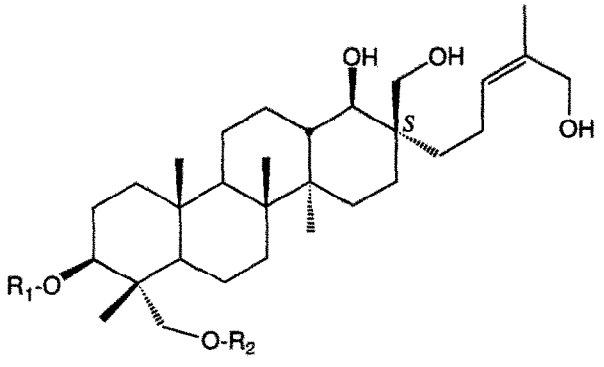


	R_1	R_2
1	-Glc^{2}Glc	-Glc
4	-Glc	-Glc
6	-H	-H
7	-Glc	-H

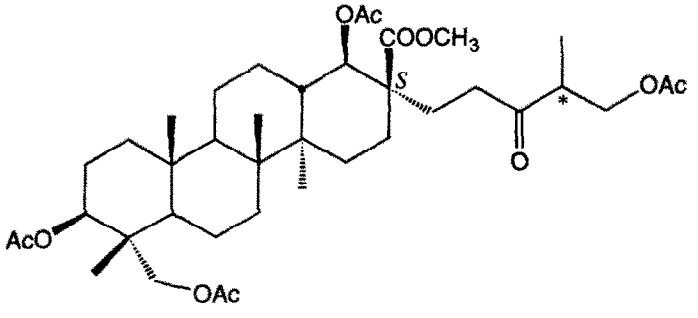
Glc=β-D-glucopyranosyl



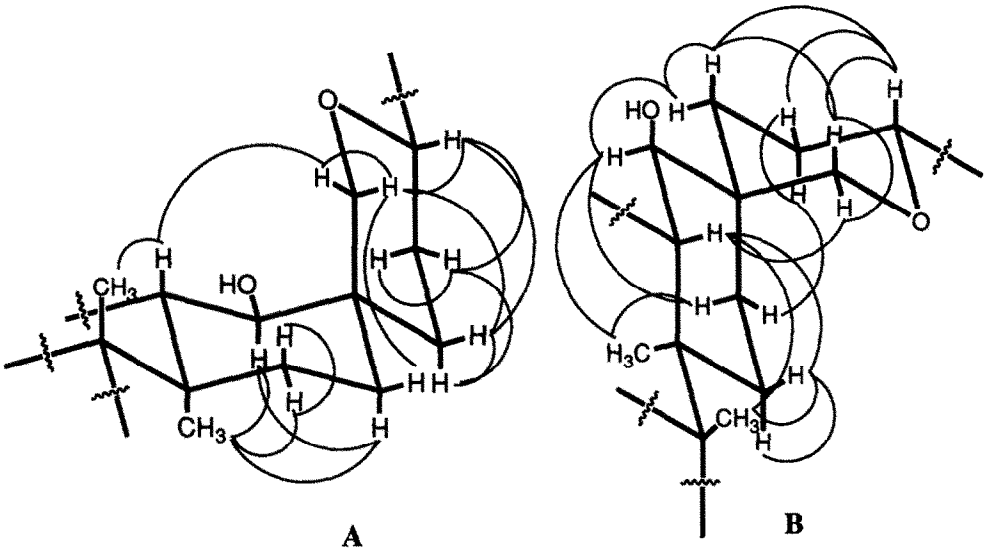
	R_1	R_2
2	-Glc^{2}Glc	-Glc
5	-Glc^{2}Glc	-H
8	-H	-H
9	-Glc	-H



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



	*
6c	*S*
8c	*R*



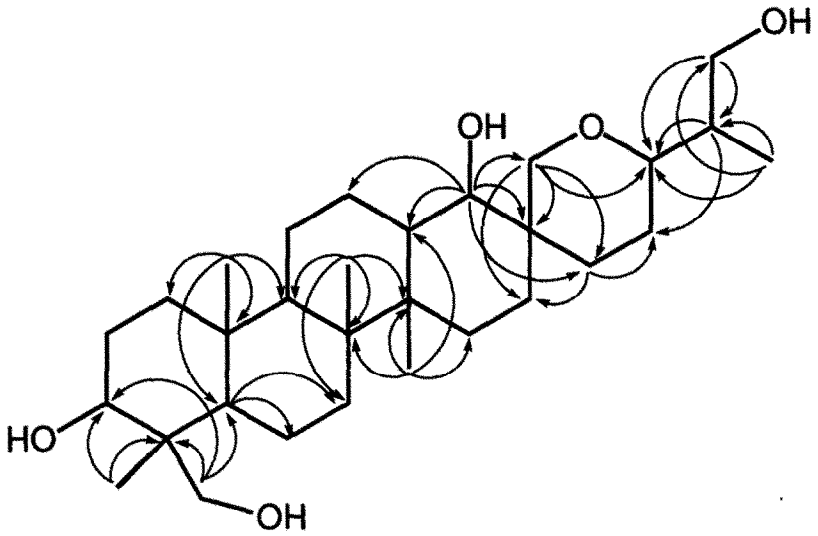


Fig. 1 ^{1}H-^{13}C long range couplings of **8**

Hosenkoside D (**4**), one of the minor saponins, mp 241-243°C, $[\alpha]_D^{20}$ +16.9° (py), has the molecular, $C_{42}H_{72}O_{15}\cdot 5/2H_2O$ based on the elementary analysis. On acid hydrolysis, **4** afforded **6** and D-glucose confirmed by specific rotation using chiral detection in HPLC. The ^{1}H- and ^{13}C- NMR spectra of **4** indicated the

Table 2. ^{13}C-NMR data for compounds **1**-**6**, **6c**, **7**, **8**, **8c**, **9**-**12** (Py-d_5, δ-values)

C no.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]	**6**[b]	**6c**[b]	**7**[b]	**8**[b]	**8c**[b]	**9**[b]	**10**[b]	**11**[b]	**12**[b]
1	38.8	39.1	38.7	38.8	39.0	39.1	38.0	39.1	39.1	38.0	39.1	38.8	39.2	39.1
2	26.6	26.2	26.6	26.4	26.1	27.8	24.6	26.1	27.8	24.6	26.1	26.4	26.2	27.9
3	81.9	82.6	81.8	81.6	82.6	73.3	74.4	82.0	73.3	74.4	81.9	81.7	82.2	73.3
4	43.3	43.6	43.2	43.2	43.6	43.0	40.9*	43.6	43.0	40.9*	43.6	43.2	43.7	43.0
5	47.8	48.3	47.8	47.8	48.4	48.8	48.6	48.0	48.8	48.5	47.8	47.8	48.0	48.8
6	17.9	18.2	17.9	17.8	18.2	18.5	18.1	18.2	18.5	18.1	18.1	17.9	18.3	18.5
7	33.7	33.7	33.7	33.6	33.6	33.6	33.6	33.6	33.6	33.6	33.6	33.6	33.8	33.6
8	40.8	41.0	40.9	40.8	41.0	40.9	41.0*	40.8	41.0	41.0*	41.0	40.9	41.1	41.0
9	51.1	51.2	51.0	51.0	51.2	51.3	50.7	51.0	51.2	50.7	51.2	51.0	51.4	51.2
10	36.9	37.0	36.9	36.9	36.9	37.3	37.1	36.9	37.3	37.1	37.0	36.9	37.2	37.3
11	21.2	21.2	21.2	21.2	21.2	21.3	21.0	21.2	21.2	21.0	21.2	21.2	21.2	21.3
12	24.9	25.6	25.3	24.9	25.6	24.8	23.4	24.9	25.6	23.4	25.6	25.3	25.5	25.3
13	41.8	39.9	40.6	41.8	39.9	41.7	38.7	41.8	39.9	38.7	39.9	40.6	40.6	40.5
14	42.2	42.2	42.5	42.1	42.2	42.2	42.2	42.2	42.2	42.2	42.2	42.5	42.7	42.5
15	26.8	26.4	26.7	26.7	26.4	26.7	27.7	26.7	26.4	27.7	26.4	26.7	26.9	26.7
16	32.9	26.5	28.2	32.9	26.6	32.9	29.9	32.9	26.6	29.2	26.6	28.2	28.4	28.3
17	79.9	76.1	77.3	79.8	76.1	79.8	77.0	79.8	76.0	77.0	76.1	77.3	77.4	77.3
18	15.7	15.9	15.7	15.6	15.8	15.8	15.8	15.6	15.9	15.8	15.9	15.7	16.0	15.9
19	17.4	17.1	17.3	17.3	17.0	17.0	16.7	17.4	16.9	16.8	17.1	17.3	17.1	17.0
20	36.0	38.4	41.5	35.9	38.4	35.9	50.4	35.9	38.4	50.4	38.4	41.5	41.7	41.5
21	72.6	67.7	65.0	72.5	67.8	72.4	174.0	72.6	67.8	174.0	67.8	65.0	64.7	65.0
22	37.7	34.4	38.0	37.6	34.5	37.6	31.3	37.4	34.5	31.3	34.5	38.0	38.1	38.0
23	26.1	24.5	22.0	26.0	24.8	26.0	36.9	26.0	24.9	36.8	24.9	22.0	22.0	21.9
24	79.7	79.7	127.9	79.7	80.6	79.6	210.7	79.7	80.6	210.7	80.6	127.9	128.1	127.8
25	40.6	39.5	136.0	40.6	41.9	40.5	45.6	40.5	41.9	45.5	41.9	136.0	136.1	136.0
26	64.4	72.5	60.9	64.5	65.0	64.4	65.8	64.3	65.0	65.8	64.9	60.9	61.0	60.8
27	13.5	13.7	21.9	13.5	13.5	13.4	13.5	13.5	13.5	13.5	13.6	21.9	22.0	21.8
28	71.8	64.9	72.0	71.8	65.0	67.8	65.6	64.5	67.8	65.5	64.5	71.8	64.7	67.8
29	13.4	13.3	13.3	13.3	13.2	12.9	13.0	13.4	12.9	13.0	13.4	13.4	13.6	12.9
30	15.1	15.2	15.2	15.0	15.2	14.9	14.5	15.0	15.2	14.6	15.2	15.1	15.2	15.0
3-*O*-Glc														
1	104.6	103.9	104.6	105.8	103.9			105.9			105.8	106.1	105.9	
2	83.8	84.0	83.8	75.3	84.1			75.3			75.8	75.9	76.0	
3	78.2	78.0	78.1	78.6	78.0			78.5			78.6	78.6	78.8	
4	71.9	71.3	71.9	71.8	71.3			71.7			71.7	71.8	71.8	
5	78.1	78.4	78.0	78.3	78.3			78.2			78.3	78.3	78.4	
6	63.0	62.7	63.0	63.0	62.7			63.0			62.9	63.0	63.0	
1'	106.1	105.9	106.2		106.0									
2'	77.1	76.8	77.4		76.8									
3'	78.5	78.4	78.5		78.4									
4'	71.7	71.3	71.5		71.3									
5'	77.7	78.0	77.7		78.0									
6'	62.8	62.6	62.8		62.5									
26 or 28-*O*-Glc														
1	105.3	105.0	105.4	106.1								105.4		
2	75.5	75.2	75.5	75.9								75.3		
3	78.5	78.6	78.5	78.7								78.7		
4	71.9	71.7	72.0	72.0								72.0		
5	77.9	78.3	78.0	77.9								77.9		
6	63.1	62.7	63.0	63.2								63.2		

(a) Obtained on a Varian UNITY 600; (b) Obtained on a JEOL GX-400; *Assignments may be interchanged in each column.

presence of two β-glucopyranosyl units [H-1:δ 5.56 (d, *J*=7.8 Hz), C-1:δ 105.8, H-1:δ 5.29 (d, *J*=7.8 Hz), C-1:δ 106.1]. A crude cellulase treatment of **4** gave a presapogenin I (**7**) and **6**.

Presapogenin I (**7**), mp 285-287°C, $[\alpha]_D^{20}$ +37.9°(py) revealed a quasi-molecular ion peak at *m/z* 653 $[M-H]^-$ in the negative FABMS, suggesting that **7** was a monoglucoside. Comparison of ^{13}C-NMR spectrum of **7** with that of **6** showed that the chemical shifts at the C-2 and C-3 in **7** were shifted[7,8] by -1.8 ppm and +8.7 ppm, respectively, indicating a β-glucopyranosyl group being joined to the C-3-OH. Therefore, **7** was formulated as hosenkol A 3-*O*-β-D-glucopyranoside. Comparison of ^{13}C-NMR spectrum of **4** with that of **7** showed a glycosylation shift for the C-28 signal (+7.3 ppm), demostrating a β-glucopyranosyl group is located at the C-28-OH. Therefore, **4** was formulated as hosenkol A 3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside.

Hosenkoside A (**1**), one of the major saponins, mp 234-236°C, $[\alpha]_D^{20}$ +20.9° (py), has the molecular, $C_{48}H_{82}O_{20}\cdot H_2O$ based on the elementary analysis. On acid hydrolysis, **1** afforded **6** and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **1** indicated the presence of three β-glucopyranosyl units [H-1:δ 5.68 (d, *J*=8.2 Hz), C-1:δ 104.6, H-1':δ 5.36 (d, *J*=7.2 Hz), C-1':δ 106.1, H-1:δ 5.28 (d, *J*=7.2 Hz), C-1:δ 105.3]. A crude cellulase treatment of **1** gave **4**, **6** and **7**. The sugar sequence of **1** was determined by HMBC experiment. The HMBC spectrum of **1** showed long-range correlations between H-1 (δ 5.68) of the inner glucose and C-3 (δ 81.9), H-1' (δ 5.36) of the outer glucose and C-2 (δ 83.8) in the inner glucose, and H-1 (δ 5.28) of the glucose and C-28 (δ 71.8), indicating a β-sophorosyl unit to be located at C-3-OH and a β-glucosyl unit to be located at C-28-OH. Hence, **1** was formulated as hosenkol A 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside.

A cellulase treatment of hosenkosides B (**2**) and E (**5**) gave hosenkol B (**8**) for aglycon, mp 265-267 °C, $[\alpha]_D^{20}$ +50.4° (py), showed a $[M+H_2O]^+$ ion peak at *m/z* 474.3705 in the HRMS suggesting the molecular formula to be $C_{30}H_{52}O_5$. The ^{13}C-NMR (INEPT) and ^{1}H-NMR (D_2O exchange) spectra indicated the presence of five methyls, ten methylenes, four methines, five quarternary carbons, three oxygen-bearing methylenes and three oxygen-bearing methines. The ^{1}H-^{1}H COSY and TOCSY spectra of **8** revealed for isolated spin systems (H-1~3, H-5~7, H-9~11~13~17, H-15~16, H-21, H-22~26). The gross structure of **8** was determined by analysis of NMR data including ^{1}H-^{1}H COSY, HMQC and HMBC experiments (Fig.1), and by referring the data of **6**. Thus, **8** is shown to be 3,17,26,28-tetrahydroxy-21,24-epoxybaccharane. The relative stereochemistry of **8** was determined by ROESY experiment (partial structure **B**). NOE experiments clearly defined the usual chair conformation and trans junction of rings A, B, C and D. Determination of the absolute configuration of C-3 in **8** was achieved by the same way to that employed for **6**. Hosenkol B 17,26-diacetate (**8b**) derived from **8** via 3, 28-*O*-isopropylidine acetal (**8a**) in the presence of Eu(fod)$_3$ showed a positively split cotton effect, $\Delta\varepsilon_{310}$+0.37, indicating the C-3 *S* configuration. The NOEs were observed between H-13β (δ 1.78) and H-21α (δ 4.07) and between H-17α (δ 3.32) and H-22α (δ 1.36) indicating the C-20 to be *S* configuration. The *R* configuration of C-24 was determined as follows: the NOE was observed between H-16β (δ 2.32) and H-23β (δ 1.73) in **8**, and H-16β (δ 2.32), and H-22β (δ 2.59) resonated at low-field due to the anisotropy of C-17-OH or epoxy oxygen, compared to H-16β (δ 1.22) and H-22α (δ 1.34) in **6**. To determine the configuration at C- 25, both acetates of **6** and **8** were oxidized with sodium periodate-rutenium chloride, followed by methylation with CH_2N_2 to give degradation products **6c**

and **8c**, respectively. Both the ^{13}C-NMR spectra of compounds **6c** and **8c** were superimposable (less than 0.1 ppm), supporting the configuration at C-20 in both compounds to be same. Their ^{1}H-NMR spectra showed the difference in side chain: H-23 appeared as double triplet at δ 2.85 and 2.75, and H-25 appeared as a quartet at δ 3.03 in **6c**, meanwhile the former appeared as unresolved multiplet for 2H at δ 2.81 and the latter appeared as a quartet at δ 3.05 in **8c**, confirming the configuration at C-25 in **8c** to be *R*. Hence, **8** was formulated as (3*S*,4*R*,17*R*,20*S*,24*R*,25*R*)-3,17,26,28-tetrahydroxy-21,24-epoxybaccharane.

Hosenkoside E (**5**), mp 243-245°C, $[\alpha]_D^{20}$ +26.1°(py) had the same molecular formula $C_{42}H_{72}O_{15}$ as **4**. On acid hydrolysis, **5** afforded **8** and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **5** indicated the presence of two β-glucopyranosyl units [H-1':δ 5.38 (d, *J*=7.8 Hz), C-1':δ 106.0, H-1:δ 5.10 (d, *J*=6.8 Hz), C-1:δ 103.9]. A crude cellulase treatment of **5** gave a presapogenin II (**9**) and **8**.

Presapogenin II (**9**), mp 231-234°C, $[\alpha]_D^{20}$ +30.2°(py) revealed a molecular ion peak at m/z 653 $[M-H]^-$ in the negative FABMS, suggesting that **9** was a monoglucoside. The C-3 signal in the ^{13}C-NMR spectrum of **9** appeared at lower field by 8.6 ppm than that of **8** because of the glycosylation shift, demonstrating that a β-glucopyranosyl group is located at the C-3-OH of aglycon. Therefore, **9** was determined as hosenkol B 3-*O*-β-D-glucopyranoside. Comparison of ^{13}C-NMR spectrum of **5** with that of **9** showed a glycosylation shift for the C-2 signal (+8.3 ppm) of glucosyl, demostrating a β-glucopyranosyl group is located at the C-2-OH of glucose. Therefore, **5** was formulated as hosenkol B 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside.

Hosenkoside B (**2**), one of the major saponins, mp 205-207°C, $[\alpha]_D^{20}$ +20.7° (py), had the same molecular formula $C_{48}H_{78}O_{20}$ as **1**. On acid hydrolysis, **2** afforded **6**, **8** and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of three β-glucopyranosyl units [H-1:δ 5.39 (d, *J*=7.3 Hz), C-1:δ 105.9, H-1':δ 5.12 (d, *J*=7.0 Hz), C-1':δ 103.9, H-1:δ 4.88 (d, *J*=8.1 Hz), C-1:δ 105.0]. A crude cellulase treatment of **2** gave **5**, **8** and **9**. In the same way to that employed for **9** , a glycosylation shift (**2** vs **5**) was observed for the C-26 signal (+7.5 ppm) disclosing the site of glycosylation. Hence, **2** was formulated as hosenkol B 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-26-*O*-β-D-glucopyranoside.

Hosenkoside C (**3**), one of the major saponins, mp 241-243°C, $[\alpha]_D^{20}$ +7.8° (*c* 0.9, py), has the molecular, $C_{48}H_{82}O_{20}\cdot 2H_2O$ based on the elementary analysis. On acid hydrolysis **3** afforded **8** and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **3** indicated the presence of three β-glucopyranosyl units [H-1:δ 5.31 (d, *J*=7.2 Hz), C-1:δ 105.4, H-1':δ 5.35 (d, *J*=7.2 Hz), C-1':δ 106.2, H-1:δ 5.72 (d, *J*=8.2 Hz), C-1:δ 104.6]. A crude cellulase treatment of **3** gave a presapogenin III (**10**), presapogenin IV (**11**) and hosenkol C (**12**).

Hosenkol C (**12**), mp. 173-175 °C, $[\alpha]_D^{20}$ + 43.3° (py), showed the same molecular formula $C_{30}H_{52}O_5$ as **8**. The ^{13}C-NMR (INEPT) and ^{1}H-NMR (D_2O exchange) spectra indicated the presence of five methyls, ten methylenes, three methines, five quaternary carbons, three oxygen-bearing methylenes, two oxygen-bearing methines and one double bond. The ^{1}H-^{1}H COSY and TOCSY spectra of **12** revealed for isolated spin systems (H-1~3, H-5~7, H-9~11~13~17, H-15~16, H-21H, H-22~26). The gross structure of **12** was determined by analysis of NMR data including ^{1}H-^{1}H COSY, HMQC and HMBC experiments and the fact: compound **12** gave **6** and **8** by acid treatment. The configuration of 24,25-doble bond was assigned to be Z, since an NOE was observed between H-24 (δ 5.48) and H-27 (δ 2.02). Hence, **12** was formulated as (3*S*, 4*R*,17*R*,20*S*,24*Z*)-3,17,21,26,28-pentahydroxybacchar-24-ene.

Presapogenin IV (**11**), mp 225-227°C, $[\alpha]_D^{20}$ +28.1°(py) revealed a quasi-molecular ion peak at *m/z* 653 $[M-H]^-$ in the negative FABMS, suggesting that **11** was a monoglucoside. The C-3 signal in the ^{13}C-NMR spectrum of **11** appeared at lower field by 9.9 ppm than that of **12** because of the glycosylation shift, demonstrating that a β-glucopyranosyl group is located at the C-3-OH of aglycon. Therefore, **11** was determined as hosenkol C 3-*O*- β-D-glucopyranoside.

Presapogenin III (**10**) obtained as colorless needles, had the same molecular formula $C_{42}H_{72}O_{15}$ [FABMS, *m/z* 815] as **4**. The ^1H- and ^{13}C-NMR spectra of **10** indicated the presence of two β-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), demostrating a β- glucopyranosyl group to be located at the C-28-OH. Therefore, **10** was formulated as hosenkol C 3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside. The sugar sequence of **3** was determined by HMBC experiment. The HMBC spectrum of **3** showed long-range correlations between H-1 (δ 5.72) of the inner glucose and C-3 (δ 81.8), H-1' (δ 5.35) of the outer glucose and C-2 (δ 83.8) of the inner glucose, and H-1 (δ 5.31) of the glucose and C-28 (δ 72.1), indicating a β-sophorosyl unit to be located at C-3-OH and a β-glucosyl unit to be located at C-28-OH. Hence, **3** was formulated as hosenkol C 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside.

To the best of our knowledge, hosenkosides A, B, D, and E were the first examples of 21, 24-epoxybaccharane glucosides.

EXPERIMENTAL

General Methods. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. CD spectra were determined JASCO J-500C. IR spectra were taken on a Hitachi IR-27G (KBr) and JASCO-FT/IR-5300 (film). UV spectra were recorded on Shimadzu UV-160. NMR spectra were recorded on a JEOL GX-400 or Varian UNIYT600 spectrometer in C_5D_5N solution using TMS as an internal standard. NMR experiments included ^1H-^1H-COSY, HMQC, INEPT, TOCSY, NOESY and HMBC (512 × 1024 data matrix size, 128 scans, recycle delay=1.16s). Coupling constants (*J* values) are given in hertz (Hz). The HRMS, CIMS (CH_4) and FDMS, and FABMS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as matrix) were measured on JEOL JMS-HX-100 and JEOL JMS-PX303 mass spectrometer, respectively. For column chromatography, silica gel 60 (40-63 μm, Merck) and silanised silica gel 60 (63-200 μm, Merck) was used. TLC was carried out on silica gel 60F-254 (Merck) with the following system $CHCl_3$-MeOH-H_2O (65:30:4), and silica gel 60 silanised with the following system MeOH-H_2O (1:1). For enzymatic hydrolysis, cellulase from *Aspergillus niger* (Sigma, type I) were used.

Isolation of saponins. Powdered dried seeds of *Impatiens balsamina* were defatted with n-hexane and extracted with 80% 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 buthanolic layer (60 g) was chromatographed on a silica gel column and eluted with EtOAc-MeOH (4:1) solvent system to give saponin fractions. These saponin factions (15 g) were repeatedly chromatographed on silica gel with $CHCl_3$-MeOH-H_2O (65:30:4) and silanised silica gel with MeOH-H_2O (1:1) to afford hosenkosides A (**1**, 3.0 g), B (**2**, 1.75 g), C

(**3**, 1.0 g), D (**4**, 0.050 g) and E (**5**, 0.19 g).

Hosenkoside A (**1**). Colorless prisms (MeOH), Mp 234-236°C, $[\alpha]_D^{20}$ + 20.9° (*c* 1.3, py). *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. IR ν_{max} (KBr) cm^{-1}: 3450, 1070, 1040. FDMS *m/z* 1001[M+Na]$^+$. ^{1}H-NMR (600 MHz): 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 3-Glc), 5.68 (1H, d, *J*=8.2 Hz, H-1 of 3-Glc). For ^{13}C-NMR data, see Table 2.

Hosenkoside B (**2**). Colorless prisms (MeOH), Mp 205-207°C, $[\alpha]_D^{20}$ + 20.7° (*c* 1.0, py). *Anal.* Calcd for $C_{48}H_{82}O_{20}\cdot H_2O$: C, 57.81; H, 8.49. Found: C, 57.85; H, 8.65. IR ν_{max} (KBr) cm^{-1}: 3400, 1070, 1040. FABMS *m/z* 977 [M-H]$^-$. ^{1}H-NMR (400 MHz): δ 0.83, 0.91, 0.95, 1.11 (3H each, s, *tert*-CH_3), 1.10 (3H, d, *J*=6.0 Hz, H-27), 2.20 (1H, m, H-25), 3.32 (1H, d, *J*=10.2 Hz, H-17), 3.76, 4.18 (each 1H, d, *J*=10.2 Hz, H-28), 4.00, 4.29 (each 1H, d, *J*=10.7 Hz, H-21), ca 4.40 (1H, m, H-3), 4.88 (1H, d, *J*=8.1 Hz, H-1 of 26-Glc), 5.12 (1H, d, *J*=7.0 Hz, H-1 of 3-Glc), 5.39 (1H, d, *J*=7.3 Hz, H-1' of 3-Glc). For ^{13}C-NMR data, see Table 2.

Hosenkoside C (**3**). Colorless prisms ($MeOH/H_2O$), Mp 241-243°C, $[\alpha]_D^{20}$ + 7.8° (*c* 0.9, py). *Anal.* Calcd for $C_{48}H_{82}O_{20}\cdot 2H_2O$: C, 56.79;H, 8.54. Found: C, 56.64; H, 8.50. IR ν_{max} (KBr) $cmcm^{-1}$: 3400, 1075, 1035. FDMS *m/z* 1001[M+Na]$^+$. ^{1}H-NMR (600 MHz): δ 0.82, 0.86, 0.89, 1.05, 2.02 (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), 4.23, 4.54 (each 1H, d, *J*=10.5 Hz, H-28), ca 4.45 (1H, m, H-3), 4.51, 4.53 (each 1H, d, *J*=11.0 Hz, H-26), 5.31 (1H, d, *J*=7.2 Hz, H-1 of 28-Glc), 5.35 (1H, d, *J*=7.2 Hz, H-1' of 3-Glc), 5.48 (1H, t, *J*=6.8 Hz, H-24), 5.72 (1H, d, *J*=7.3 Hz, H-1 of 3-Glc). For ^{13}C-NMR data, see Table 2.

Hosenkoside D (**4**). Colorless prisms (MeOH), Mp 241-243°C, $[\alpha]_D^{20}$ + 16.9° (*c* 1.1, py). *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. IR ν_{max} (KBr) $cmcm^{-1}$: 3450, 1070, 1040. FABMS *m/z* 815 [M-H]$^-$. ^{1}H-NMR (400 MHz): 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 28-Glc), 5.56 (1H, d, *J*=7.8 Hz, H-1 of 3-Glc). For ^{13}C-NMR data, see Table 2.

Hosenkoside E (**5**). Colorless prisms (MeOH), Mp 243-245°C, $[\alpha]_D^{20}$ + 26.1° (*c* 0.85, py). *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot 3/2H_2O$: C, 59.77; H, 8.96. Found: C, 59.95; H, 8.84. IR ν_{max} (KBr) $cmcm^{-1}$: 3400, 1070, 1040. FABMS *m/z* 815 [M-H]$^-$. ^{1}H-NMR (400 MHz): δ 0.84, 0.92, 0.96, 1.10 (3H each, s, *tert*-CH_3), 1.15 (3H, d, *J*=6.8 Hz, H-27), 2.10 (1H, m, H-25), 3.33 (1H, d, *J*=10.3 Hz, H-17), 3.70, 4.20 (each 1H, d, *J*=11.0 Hz, H-28), 4.10, 4.30 (each 1H, d, *J*=11.0 Hz, H-21), ca 4.20 (1H, m, H-3), 5.10 (1H, d, *J*=6.8 Hz, H-1 of 3-Glc), 5.38 (1H, d, *J*=7.8 Hz, H-1' of 3-Glc). For ^{13}C-NMR data, see Table 2.

Enzymatic hydrolysis of hosenkoside A (1). To a solution of hosenkoside A (1 g) in EtOH (26 ml) and 0.01M NaH_2PO_4 buffer (pH4.0, 190 ml) incubated with crude cellulase (1 g, Sigma) for two days at

37°C and work-up as usual. The crude product was chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (25:8:0.1) giving hosenkol A (**6**, 220 mg), presapogenin I (**7**, 120 mg) and hosenkoside D (**4**,80 mg). Compound **6**, colorless prisms (MeOH), mp 225-227°C, $[\alpha]_D^{20}$ + 78.9° (c 1.51, py). HREIMS obsd for $C_{30}H_{52}O_5$ 474.3705, calcd 474.3709. IR ν_{max} (KBr) cm^{-1}: 3500, 3400, 1050, 1030. For NMR data, see Tables 1 and 2. Compound **7**, colorless prisms (MeOH), mp 285-287°C, $[\alpha]_D^{20}$ + 37.9° (*c* 0.7, py). FABMS *m/z* 653 $[M-H]^-$. (Found: C, 64.52; H, 9.63. $C_{36}H_{62}O_{10}H_2O$ requires: C, 64.26; H, 9.59. ^{1}H-NMR (400 MHz): 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 2.

Enzymatic hydrolysis of hosenkoside D (2). Enzymatic hydrolysis of **2** (30 mg) was carried out in the same way as **1** to give **6** (10 mg) and **7** (5 mg).

Acid hydrolysis of hosenkoside A (1). A solution of **1** (100 mg) in 5% H_2SO_4 in 50% EtOH was heated at 100°C for 3hr. 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 A (**6**, 45 mg). The aqueous layer was neutralized with Amberlite IR-45 and evaporated *in vacuo* to dryness. The form (D or L) of each sugar was determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1), respectively in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70°C) by comparison with authentic sugars (10 mM each of D-glc and L-glc). The sugar part gave a peak indicating positive optical rotation at 7.38 min (D-glc, 7.36 min).

Acid hydrolysis of hosenkoside D (4). Acid hydrolysis of **4** (10 mg) was carried out in the same way as for **1** to give **6** (5 mg) as well as D-glc.

Acetalizatioin of hosenkol A (6). To a solution of hosenkol A (50 mg) in CH_2Cl_2 (5 ml) and 2, 2-dimethoxypropane (3 ml) was added *p*-toluenesulphonic acid monohydrate (10 mg), and the mixture was stirred at room temperature for 1 hr. After addition of water, the product was extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated to dryness. The crude product was subjected to silica gel column chromatography with $CHCl_3$-MeOH (99:1) to give isopropylidene acetal of **6** (**6a**, 45 mg). Compound **6a**, powder, $[\alpha]_D^{20}$ + 34.6° (*c* 1.8, MeOH). HRCIMS *m/z* 533.4198, CIMS *m/z* 533 $[M+H]^+$, 518, 474, 458, 440, 427, 415, 399, 397. IR ν_{max} (film) cm^{-1}: 3480, 1255, 1205, 1100, 1065, 862. ^{1}H-NMR (400 MHz) δ: 0.84, 0.90, 0.90, 1.16 (3H each, s, *tert*-CH_3), 1.10 (3H, d, *J*=6.6 Hz, H-27), 2.13 (1H, m, H-25), 3.30, 4.59 (each 1H, d, *J*=11.7 Hz, H-21), 3.45 (1H, d, *J*=10.0 Hz, H-17), 3.53, 3.64 (each 1H, d, *J*=10.3 Hz, H-28), 3.55 (1H, ddd, *J*=11.0, 10.0, 3.0 Hz, H-24), 3.64 (1H, dd, *J*=11.0, 3.5 Hz, H-3), 3.87 (1H, dd, *J*=10.3, 4.5 Hz, H-26), 4.01 (1H, dd, *J*=10.3, 3.5 Hz, H-26). ^{13}C-NMR (100 MHz) δ: 12.7 (C-29), 13.5 (C-27), 14.9 (C-30), 15.6 (C-18), 17.8 (C-19), 19.6 (C-6), 19.6 ($O_2C(\underline{C}H_3)_2$), 21.0 (C-11), 24.2 (C-2), 24.8 (C-12), 26.0 (C-23), 26.7 (C-15), 30.3 ($O_2C(\underline{C}H_3)_2$), 32.9 (C-16), 33.3 (C-7), 36.0 (C-20), 37.0 (C-10), 37.6 (C-4), 37.6 (C-22), 39.3 (C-1), 40.5 (C-25), 41.1 (C-8), 41.7 (C-13), 42.2 (C-14), 51.1 (C-5), 51.7 (C-9), 64.4 (C-26), 72.4 (C-21 or C-28), 72.5 (C-28 or C-21), 77.6 (C-3), 79.6 (C-17), 79.7 (C-24), 98.9 ($O_2\underline{C}(CH_3)_2$).

Acetylation of acetonide of hosenkol A and removing the isppropylidene group. Treatment of 40 mg of **6a** with 1 ml of acetic anhydride in 1 ml of pyridine with stirring at 37°C for 3 hr followed by work-up gave diacetate of **6a** (40 mg). Diacetate of **6a**, oil, $[\alpha]_D^{20}$ + 24.8° (*c* 3.0, MeOH), HRCIMS obsd for $[C_{37}H_{60}O_7+H]$ 617.4396, calcd.617.4418, CIMS *m/z* 617 $[M+H]^+$. IR ν_{max} (film) cm^{-1}: 1740, 1245, 1105, 1065, 1030, 862. ^{1}H-NMR (400 MHz) δ: 0.80, 0.86, 0.93, 1.15 (3H each, s, *tert*-CH_3), 1.15 (3H, d, *J*=6.6 Hz, H-27), 3.26 (1H, ddd, *J*=11.0, 10.0, 3.5 Hz, H-24), 3.33, 4.36 (each 1H, d, *J*=12.4 Hz, H-21), 3.53, 3.63 (each 1H, d, *J*=11.0 Hz, H-28), 3.64 (1H, m, H-3), 4.16 (1H, dd, *J*=10.2, 4.5 Hz, H-26), 4.53 (1H, dd, *J*=10.2, 3.7 Hz, H-26), 5.08 (1H, d, *J*=11.0 Hz, H-17). ^{13}C-NMR (100 MHz) δ: 12.7 (C-29), 13.9 (C-27), 14.8 (C-30), 15.6 (C-18), 17.6 ($O_2C(\underline{C}H_3)_2$), 17.7 (C-19), 19.6 (C-6), 20.5 ($OCO\underline{C}H_3$), 20.8 ($OCO\underline{C}H_3$), 21.0 (C-11), 24.2 (C-2), 24.3 (C-23), 24.8 (C-12), 26.3 (C-15), 30.3 ($O_2C(\underline{C}H_3)_2$), 32.5 (C-16), 33.3 (C-7), 34.4 (C-22)36.0 (C-20), 36.0 (C-20), 36.1 (C-13), 37.0 (C-10), 37.4 (C-4), 38.0 (C-25), 391 (C-1), 41.1 (C-8), 42.6 (C-14), 50.6 (C-5), 51.5 (C-9), 66.8 (C-26), 68.6 (C-21), 72.4 (C-28), 77.4 (C-17), 77.6 (C-3), 80.0 (C-24), 99.0 ($O_2\underline{C}(CH_3)_2$). Diacetate of **6a** (35 mg) was heated in 50% AcOH solution at 60°C for 6 hr and work-up as usual. The crude products (32 mg) was subjected to Sephadex LH-20 using MeOH as solvent to afford hosenkol A 17, 26-diacetate (**6b**, 28 mg). Compound **6b**, oil, $[\alpha]_D^{20}$ + 31.9° (*c* 2.6, MeOH), HRCIMS obsd for $[C_{34}H_{56}O_7+H]$ 575.3962, calcd.575.3948, CIMS *m/z* 575 $[M+H]^+$. IR ν_{max} (film) cm^{-1}: 1245, 1105, 1065, 1030, 860. UV (2×10^{-4} M Eu(fod)$_3$-CCl_4) λ_{max} nm (log ε): 330 (log 4.47), 266 (log 5.38), CD (c 0.14, 2×10^{-4} M Eu(fod)$_3$-CCl_4) λ_{max} nm (Δ ε): 335 (0), 310 (+0.14), 296 (0), 285 (-0.17). ^{1}H-NMR (400 MHz) δ: 0.88, 0.91, 0.93, 1.05 (3H each, s, *tert*-CH_3), 4.21 (1H, dd, *J*=11.0, 5.5 Hz, H-3), 4.53 (1H, dd, *J*=11.0, 4.4 Hz, H-26), 5.07 (1H, d, *J*=11.0 Hz, H-17). ^{13}C-NMR (100 MHz) δ: 12.7 (C-29), 13.9 (C-27), 14.8 (C-30), 15.7 (C-18), 16.9 (C-19), 18.4 (C-6), 20.7 ($OCO\underline{C}H_3$), 20.8 ($OCO\underline{C}H_3$), 21.3 (C-11), 24.4 (C-23), 24.8 (C-12), 26.3 (C-15), 27.8 (C-2), 32.5 (C-16), 33.5 (C-7), 34.4 (C-22), 36.0 (C-13 or C-20), 36.1 (C-20 or C-13), 37.2 (C-10), 38.2 (C-25), 39.0 (C-1), 40.9 (C-8), 42.6 (C-14), 43.0 (C-4), 48.6 (C-5), 50.8 (C-9), 66.8 (C-26), 67.5 (C-28), 68.6 (C-21), 73.0 (C-3), 77.3 (C-17), 80.1 (C-24).

Enzymatic hydrolysis of hosenkoside B (2). Enzymatic hydrolysis of **2** (800 mg) was carried out in the same way as **1** to give hosenkoside E (**5**, 60 mg), hosenkol B (**8**, 180 mg) and presapogenin II (**9**, 100 mg). Compound **8**, colorless prisms (MeOH), mp 265-267 °C, $[\alpha]_D^{20}$ + 50.4° (c 1.14, py). HREIMS obsd for [M ($C_{30}H_{52}O_5$)-H_2O] 474.3705, calcd 474.3709. IR ν_{max} (KBr) cm^{-1}: 3500, 3400, 3260, 1080, 1045. For NMR data, see Tables 1 and 2. Compound **9**, colorless prisms (MeOH), mp 232-234°C, $[\alpha]_D^{20}$ + 30.2° (*c* 1.26, py). FABMS *m/z* 653 $[M-H]^-$. (Found: C, 65.70; H, 9.69. $C_{36}H_{62}O_{10}$ requires: C, 66.02; H, 9.54. IR ν_{max} (KBr) cm^{-1}: 3450, 1070, 1040. ^{1}H-NMR (400 MHz): 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 4.20 (1H, m, H-3), 5.17 (1H, d, *J*=7.0 Hz, H-1 of Glc). For ^{13}C-NMR data, see Table 2.

Enzymatic hydrolysis of hosenkoside E (5). Enzymatic hydrolysis of **5** (40 mg) was carried out in the same way as **2** to give **8** (15 mg) and **9** (6 mg).

Acid hydrolysis of hosenkoside D (4). Acid hydrolysis of **4** (10 mg) was carried out in the same

way as for **1** to give **6** (5 mg) as well as D-glc.

Acetalization of hosenkol B (8). Acetalization of **8** (50 mg) was carried out in the same way as **6a** to give isopropylidene acetal of **8** (**8a**, 45 mg). Compound **8a**, powder, $[\alpha]_D^{20}$ + 8.8° (*c* 2.82, MeOH). CI-HRMS *m/z* 533.4198 [($C_{33}H_{56}O_5$+H), calcd.533.4206], CIMS *m/z* 533 $[M+H]^+$, 518, 474, 458, 440, 427, 415, 399, 397. IR ν_{max} (film) cm^{-1}: 3430, 1255, 1205, 1065, 1035, 860. ^{1}H-NMR (400 MHz) δ: 0.80, 0.93, 0.98, 1.15 (3H each, s, *tert*-CH_3), 1.16 (3H, d, *J*=6.6 Hz, H-27), 2.10 (1H, m, H-25), 3.34 (1H, d, *J*=10.5 Hz, H-17), 3.48 (1H, ddd, *J*=11.0, 10.0, 3.0 Hz, H-24), 3.52, 3.64 (each 1H, d, *J*=11.0 Hz, H-28), 3.63 (1H, dd, *J*=11.0, 5.5 Hz, H-3), 3.8-3.95 (2H, m, H-26), 4.08, 4.29 (each 1H, d, *J*=11.7 Hz, H-21). ^{13}C-NMR (100 MHz) δ: 12.7 (C-29), 13.6 (C-27), 15.2 (C-30), 15.7 (C-18), 17.7 ($O_2C(\underline{C}H_3)_2$),17.8 (C-19), 19.6 (C-6), 21.0 (C-11), 24.2 (C-2), 24.9 (C-23), 25.5 (C-12), 26.4 (C-15 or C-16), 26.6 (C-16 or C-15), 30.3 ($O_2C(\underline{C}H_3)_2$), 33.4 (C-7), 34.5 (C-22), 37.0 (C-10), 37.5 (C-4), 38.4 (C-20), 39.3 (C-1), 39.8 (C-13), 41.2 (C-8), 41.9 (C-25), 42.2 (C-14), 51.0 (C-5), 51.7 (C-9), 64.9 (C-26), 67.8 (C-21), 72.5 (C-28), 76.0 (C-17), 77.7 (C-3), 80.6 (C-24), 99.0 ($O_2\underline{C}(CH_3)_2$).

Acetylation of acetonide of hosenkol B and removing the isopropylidene group. Acetylation of **8a** (40 mg) was carried out in the same way as for **6a** to give diacetate of **8a** (40 mg). Diacetate of **8a**, powder, $[\alpha]_D^{20}$ -0.96° (*c* 4.0, MeOH), HRCIMS obsd for [$C_{37}H_{60}O_7$+H] 617.4396, calcd.617.4418, CIMS *m/z* 617 $[M+H]^+$. IR ν_{max} (KBr) cm^{-1}: 1740, 1235, 1065, 1020, 965, 860. ^{1}H-NMR (400 MHz) δ: 0.79, 0.90, 0.95, 1.15 (3H each, s, *tert*-CH_3), 0.98 (3H, d, *J*=6.6 Hz, H-27), 3.10 (1H, ddd, *J*=11.0, 10.0, 3.5 Hz, H-24), 3.53, 3.63 (each 1H, d, *J*=11.7 Hz, H-28), 3.56, 4.16 (each 1H, d, *J*=11.7 Hz, H-21), 3.63 (1H, dd, *J*=11.0, 5.5 Hz, H-3), 4.17 (1H, dd, *J*=11.0, 5.5 Hz, H-26), 4.17 (1H, dd, *J*=11.0, 3.0 Hz, H-26), 4.95 (1H, d, *J*=11.0, H-17). ^{13}C-NMR (100 MHz) δ: 12.7 (C-29), 13.6 (C-27), 14.8 (C-30), 15.6 (C-18), 17.7 ($O_2C(\underline{C}H_3)_2$), 17.7 (C-19), 19.6 (C-6), 20.4 (C-11), 20.8 ($OCO\underline{C}H_3$), 20.8 ($OCO\underline{C}H_3$), 24.2 (C-2), 24.6 (C-16), 24.9 (C-23), 25.9 (C-12), 26.2 (C-15), 30.3 ($O_2C(\underline{C}H_3)_2$), 33.3 (C-7), 34.0 (C-22), 37.0 (C-10), 37.4 (C-13), 37.5 (C-4), 38.4 (C-20), 39.1 (C-1), 41.1 (C-8), 42.6 (C-14), 51.6 (C-5), 51.5 (C-9), 66.6 (C-26), 67.6 (C-21), 72.4 (C-28), 77.5 (C-3), 78.6 (C-17), 79.5 (C-24), 99.0 ($O_2\underline{C}(CH_3)_2$). Removing the protection group of diacetate of **8a** (35 mg) with acid was carried out in the same way as for acetate of **6a** to give hosenkol B 17, 26-diacetate (**8b** 30 mg). Compound **8b**, oil, $[\alpha]_D^{20}$ +5.5° (*c* 3.0, MeOH), HRCIMS obsd for [$C_{34}H_{56}O_7$+H] 575.3962, calcd.575.3948, EIMS *m/z* 577 $[M+H]^+$. IR ν_{max} (film) cm^{-1}: 1290, 1160, 960, 940. UV (2×10^{-4} M Eu(fod)$_3$-CCl_4) λ_{max} nm (log ε): 330 (log 4.43), 266 (log 5.28), CD (c 0.075, 2×10^{-4} M Eu(fod)$_3$-CCl_4) λ_{max} nm (Δ ε). 330 (0), 310 (+0.37), 296 (0), 285 (-0.44). ^{1}H-NMR (400 MHz) δ: 0.90, 0.90, 0.96, 1.05 (3H each, s, *tert*-CH_3), 0.98 (3H, d, *J*=7.6 Hz, H-27), 3.10 (1H, ddd, *J*=11.0 Hz, 3.0 H-24), 3.52, 3.64 (each 1H, d, *J*=11.0 Hz, H-28), 3.30, 4.59 (each 1H, d, *J*=11.7 Hz, H-21), 3.48 (1H, ddd, *J*=11.0, 10.0, 3.0 Hz, H-24), 3.57 (1H, d, *J*=11.7 Hz, H-21), 3.71 (1H, d, *J*=12.2 Hz, H-28), 4.10-4.25 (4H, m, H-3, H-21, H-26, H-28), 4.49 (1H, dd, *J*=11.0, 4.2 Hz, H-26), 4.94 (1H, d, *J*=11.0 Hz, H-17). ^{13}C-NMR (100 MHz) δ: 13.0 (C-29), 13.6 (C-27), 14.8 (C-30), 15.8 (C-18), 16.9 (C-19), 18.4 (C-6), 20.8 ($OCO\underline{C}H_3$), 20.8 ($OCO\underline{C}H_3$), 24.6 (C-16), 25.0 (C-23), 26.0 (C-12), 26.2 (C-15), 27.8 (C-2), 33.5 (C-7), 34.0 (C-22), 37.2 (C-10), 37.5 (C-13 or C-25), 37.6 (C-25 or C-13), 38.4 (C-20), 39.0 (C-1), 40.9 (C-8), 42.6 (C-14), 43.0 (C-4), 48.6 (C-5), 50.8 (C-9), 66.6 (C-26), 67.5 (C-21), 67.6 (C-28), 73.0 (C-3), 77.7 (C-3), 78.7 (C-17), 79.5 (C-24).

Acid hydrolysis of hosenkoside E (5). Acid hydrolysis of **5** (10 mg) was carried out in the same way as **1** to give **8** (5 mg) as well as D-glc.

Acid hydrolysis of hosenkoside B (2). Acid hydrolysis of **2** (10 mg) was carried out in the same way as **1** to give **6** (5 mg) as well as D-glc.

Acetylation of hosenkol A (6). To a solution of **6** (200 mg) in pyridine (5 ml) was added Ac_2O (5 ml) and left at room temp. for 24 hr and work-up as usual. The crude products (220 mg) was subjected to Sephadex LH-20 using MeOH as solvent to afford Hosenkol A tetraacetate (200 mg). Colorless prisms (MeOH), mp 213-215°C, FABMS *m/z* 649 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 1660, 1290, 1160, 960, 940. ^{1}H-NMR (400 MHz): δ 0.81, 0.84, 0.92, 0.97 (3H, each, s, *tert*-CH_3), 0.98 (d, *J*=7.0 Hz, H-27), 2.04, 2.04, 2.04, 2.21 (3H, each, s, ,Ac), 3.09 (ddd, *J*=7.0, 7.0, 3.5 Hz H-24), 3.56, 4.16 (each, d, *J*=11.2 Hz, H-21), 3.99, 4.01 (each, d, *J*=11.2 Hz, H-28), 4.18 (dd, *J*=10.8, 5.0 Hz, H-26), 4.48 (dd, *J*=10.8, 4.4 Hz, H-26), 4.95 (d, *J*=11.2 Hz, H-17), 5.04 (dd, *J*=11.2, 4.9 Hz, H-3).

Acetylation of hosenkol B (8). Acetylation of **8** (200 mg) by the same procedure as **6** gave hosenkol B tetraacetate (200 mg). FABMS *m/z* 649 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 1660, 1285, 1160, 960, 935. ^{1}H-NMR (400 MHz): δ 0.81, 0.84, 0.92, 0.97 (3H, each, s, *t ert*-CH_3), 0.99 (d, *J*=7.0 Hz, H-27), 2.04, 2.04, 2.05, 2.22 (3H, each, s, Ac), 3.27 (ddd, *J*=7.0, 7.0, 3.5 Hz H-24), 3.45, 4.39 (each, d, *J*=11.5 Hz, H-21), 4.00, 4.03 (each, d, *J* =11.2 Hz, H-28), 4.18 (dd, *J*=10.7, 8.0 Hz, H-26), 4.60 (dd, *J*=10.7, 4.4 Hz, H-26), 5.05 (dd, *J*=11.2, 5.0 Hz, H-3), 5.10 (d, *J*=11.7 Hz, H-17).

Oxidation of hosenkol A tetraacetate and methylation of oxidation product A. To a solution of Hosenkol A tetraacetate (160 mg) in CCl_4 (2 ml), CH_3CN (2 ml) and H_2O (3 ml) were added sodium methaperiodate (877 mg) and anhydrous $RuCl_3$ (5 mg), and the mixture was stirred at room temperature for 12 hr. After addition of water, the products was extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated to dryness. The crude product was subjected to silica gel column chromatography with $CHCl_3$-MeOH (99:1) to give oxidation product A (60 mg) as a glass. FABMS *m/z* 690 $[M-H]^-$. To a solution of oxidation product A (50 mg) in $CHCl_3$ (10 ml) was added etheral CH_2N_2 at the room temperature for 2 hr and worked up as usual to give methyl ester (**6c**, 50 mg) of oxidation product A as a glass. FABMS *m/z* 704 $[M-H]^-$. IR ν_{max}($CHCl_3$) cm^{-1}: 1740, 1250, 1220, 1040. ^{1}H-NMR (400 MHz): δ 0.81, 0.83, 1.01, 1.03 (3H, each, s, *tert*-CH_3), 1.12 (d, *J*= 6.8 Hz, H-27), 2.01, 2.05, 2.05, 2.12 (3H, each, s, Ac), 2.75, 2.85 (each, dt, *J*=12.0, 6.0 Hz, H-23), 3.03 (sextet, *J*=6.0 Hz, H-25), 3.78 (3H, s, $COOCH_3$), 3.99, 4.01 (each, d, *J*=11.2 Hz, H_{28}), 4.26 (dd, *J*=11.2, 5.4 Hz, H-26), 4.40 (dd, *J*=11.2, 7.0 Hz, H-26), 5.05 (dd, *J*=11.2, 5.0 Hz, H-3), 5.18 (d, *J*=11.2 Hz, H-17). ^{13}C-NMR: δ 20.6, 20.7, 20.7, 20.8 (Me-CO), 170.3, 170.5, 170.6, 171.0 (Me-CO), 51.6 (COOMe). For ^{13}C-NMR data of genin part, see Table 2.

Oxidation of hosenkol B tetraacetate and methylation of oxidation product B. Oxidation of hosenkol B tetraacetate (160 mg) by the same procedure as hosenkol A tetraacetate gave oxidation product B (85 mg) as a glass. FABMS *m/z* 690 $[M-H]^-$. Methylation of oxidation product B (60 mg) by the same

procedure as oxidation product A gave methyl ester (**8c**, 60 mg) of oxidation product B as a glass. FABMS *m/z* 704 $[M-H]^-$. IR ν_{max} ($CHCl_3$) cm^{-1}:1740, 1250, 1225, 1040. ^{1}H-NMR (400 MHz): δ0.81, 0.83, 1.00, 1.02 (3H, each, s, *tert*-CH_3), 1.10 (d, *J*=7.3 Hz, H-27), 2.02, 2.05, 2.05, 2.11 (3H, each, s, Ac), 2.81 (2H, m, H-23), 3.05 (sextet, *J*=6.0 Hz, H-25), 3.78 (3H, s, $COOCH_3$), 3.99, 4.02 (each, d, *J*=12.0 Hz, H-28), 4.28 (dd, *J*=11.2, 5.4 Hz, H-26), 4.40 (dd, *J*=11.2, 7.3 Hz, H-26), 5.02 (dd, *J*=11.2, 5.0 Hz, H-3), 5.18 (d, *J*=11.2 Hz, H-17). For ^{13}C-NMR data, see Table 2.

Acid hydrolysis of hosenkoside C (3). Acid hydrolysis of **3** (100 mg) was carried out in the same way as **1** to give **6** (20 mg) and **8** (22 mg) as well as D-glc.

Acid hydrolysis of hosenkol C (12). Acid hydrolysis of **12** (20 mg) was carried out in the same way as **1** to give **6** (8 mg) and **8** (9 mg).

Enzymatic hydrolysis of hosenkoside C (3). Enzymatic hydrolysis of **3** (200 mg) was carried out in the same way as **1** to give presapogenin III (**10**, 40 mg), presapogenin IV (**11**, 25 mg) and hosenkol C (**12**, 15 mg). Compound **10**, colorless prisms (MeOH), mp 199-201°C, $[\alpha]_D^{20}$ + 11.5° (*c* 0.66, py). *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot 3/2H_2O$: C, 59.77; H, 8.96. Found: C, 59.90; H, 8.80. IR ν_{max} (KBr) cm^{-1}: 3360, 1035. FABMS *m/z* 815 $[M-H]^-$. ^{1}H-NMR (400 MHz): 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 28-Glc), 5.50 (1H, t, *J*=6.5 Hz, H-24), 5.56 (1H, d, *J*=7.0 Hz, H-1 of 3-Glc). For ^{13}C-NMR data, see Table 2. Compound **11**, colorless prisms (MeOH), 225-227°C, $[\alpha]_D^{20}$ + 28.1° (*c* 1.14, py). *Anal.* Calcd for $C_{36}H_{62}O_{10}$: C, 66.02; H, 9.54. Found:C, 65.80; H, 9.60. IR ν_{max} (KBr) cm^{-1}: 3400, 1070, 1040. FABMS *m/z* 653 $[M-H]^-$. ^{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 3-Glc), 5.50 (1H, t, *J*=6.5 Hz, H-24). For ^{13}C-NMR data, see Table 2. Compound **12**, powder, $[\alpha]_D^{20}$ + 43.3° (*c* 0.6, py). HREIMS obsd for [M ($C_{30}H_{52}O_5$)-H_2O] 474.3705, calcd 474.3709. IR ν_{max} (KBr) cm^{-1}: 3450, 1070, 1040. For ^{13}C-NMR data, see Tables 1 and 2.

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