Structures of Five Hydroxylated Sterols from the Seeds of *Trichosanthes kirilowii* MAXIM.

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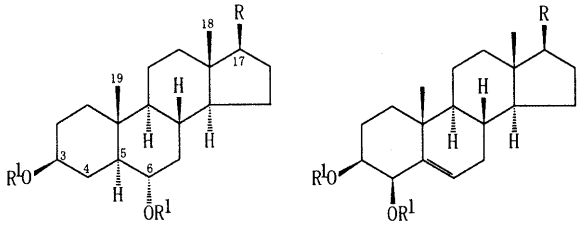
Five hydroxylated sterols, stigmastane-3β,6α-diol, poriferastane-3β,6α-diol, stigmast-5-ene-3β,4β-diol, poriferast-5-ene-3β,4β-diol, and poriferasta-5,25-diene-3β,4β-diol, the latter four of which are new naturally occurring compounds, were isolated from the unsaponifiable lipid of the seed extract of *Trichosanthes kirilowii* MAXIM. The structures were determined by spectral and chemical methods.

Key words *Trichosanthes kirilowii*; seed; hydroxylated sterol

The seeds of *Trichosanthes kirilowii* MAXIM. (Cucurbitaceae) have been used in Chinese medicine as an anti-inflammatory agent, a cough medicine, and an expectorant.[1] In previous papers, we reported the isolation and structural elucidation of ten novel triterpenes: nine D:C-friedo-oleananes[2,3] and one cucurbitane.[4] These triterpenes showed marked inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation in mice.[3–5] This paper describes how our continued study on the unsaponifiable lipid of the *T. kirilowii* seed extract led to the isolation of three mixtures of hydroxylated sterols, **A**, **B**, and **C**, and their characterization as a mixture of stigmastane-3β,6α-diol (**1a**) and poriferastane-3β,6α-diol (**1b**), a mixture of stigmast-5-ene-3β,4β-diol (**3a**) and poriferast-5-ene-3β,4β-diol (**3b**), and poriferasta-5,25-diene-3β,4β-diol (**3c**), respectively, the latter four being novel naturally occurring compounds.

Three mixtures of hydroxylated sterols **A**, **B**, and **C** were isolated as the acetyl derivatives from the unsaponifiable lipid of the seed extract of *T. kirilowii* by silica gel column chromatography followed by acetylation of the separated highly-polar fraction, and subsequent reverse phase HPLC of the acetate fraction.

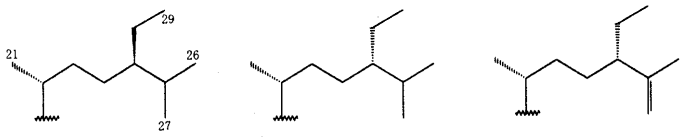
The molecular formula of the peracetate of **A** (**A**-acetate) was determined as $C_{33}H_{56}O_4$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z* 516.4175 (M^+)]. Alkaline hydrolysis of **A**-acetate yielded a free sterol mixture **A** which showed M^+ at *m/z* 432.3946 ($C_{29}H_{52}O_2$) in the HR-MS. **A** possesses a saturated 24-ethylcholestane (stigmastane and/or poriferastane) structure with two secondary hydroxyl groups, as indicated by its ^{13}C- and ^1H-NMR data (Tables 1 and 2). The hydroxymethine signals at δ 3.42 (dt, *J*=4.4, 11.0 Hz) and 3.58 (tt, *J*=5.1, 11.0 Hz) contained large *J* values (11.0 Hz), and thereby both the secondary hydroxyl groups should have equatorial configurations. The coupling patterns of the two methine signals, δ 3.42 and 3.58, suggested that these methines are incorporated into the substructures >CH–CH(eq. OH)–CH_2– and –CH_2–CH(eq. OH)–CH_2–, respectively. These and the angular methyl ^1H signals at δ 0.65 (H-18) and 0.82 (H-19), and moreover the prominent mass fragmentations at *m/z* 249 ($C_{16}H_{25}O_2^+$, ring D cleavage+1H), 231 (*m/z* 249−H_2O), and 213 (*m/z* 231−H_2O), are consistent with a hydroxylated sterol



1 R^1 = H
2 R^1 = Ac

3 R^1 = H
4 R^1 = Ac

Side chain (R)



a (24*R*) b (24*S*) c (24*S*)

Chart 1

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Table 1. ^{13}C-NMR Data (δ/ppm) for Three Mixtures of Hydroxylated Sterols A—C and Their Diacetyl Derivatives from *Trichosanthes kirilowii* Seeds ($CDCl_3$, 100.62 MHz)

^{13}C	A[a]				B[a]				C
	Diol		Diacetate		Diol		Diacetate		Diacetate
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**4c**
1	37.3		36.9		36.9		36.8		36.9
2	31.1		27.2		25.5		22.5		22.5
3	71.3		73.2		72.5		72.9		72.9
4	32.3		28.4		77.3		76.0		76.0
5	51.7		48.5		142.8		138.2		138.2
6	69.5		72.4		128.8		131.7		131.7
7	41.7		37.7		32.1		32.0		32.0
8	34.3		34.1		31.8		31.6		31.6
9	53.8		53.6		50.2		50.2		50.2
10	36.3		36.6		36.0		36.1		36.1
11	21.2		21.1		20.6		20.5		20.5
12	39.8		39.7		39.7		39.6		39.6
13	42.6		42.6		42.3		42.3		42.3
14	56.2		56.1		56.9		56.8		56.8
15	24.2		24.1		24.3		24.2		24.2
16	28.2		28.2		28.2		28.2		28.1
17	56.1		56.1		56.0		55.9		56.0
18	12.0		12.0		11.9		11.8		11.8
19	13.5		13.3		21.1		20.4		20.4
20	36.1	36.3	36.1	36.3	36.1	36.3	36.1	36.3	35.5
21	18.7	18.8	18.7	18.8	18.8		18.8		18.6
22	33.9		33.9		33.9		33.9		33.7
23	26.1	26.4	26.2	26.5	26.1	26.4	26.1	26.4	29.4
24	45.8	46.1	45.9	46.1	45.8	46.1	45.8	46.1	49.5
25	29.1	28.9	29.2	29.0	29.2	29.0	29.1	28.9	147.6
26	19.8	19.6	19.8	19.6	19.8	19.6	19.8	19.6	17.8
27	19.0		19.1	19.0	19.1	19.0	19.0		111.4
28	23.1	23.0	23.1	23.0	23.1	23.0	23.1	23.0	26.5
29	12.0	12.3	12.0	12.3	12.0	12.3	12.0	12.3	12.1
3β-OCOMe	—		21.4		—		21.5		21.5
4β-OCOMe	—		—		—		21.1		21.1
6α-OCOMe	—		21.3		—		—		—
3β-OCOMe	—		170.9		—		170.2		170.2
4β-OCOMe	—		—		—		170.3		170.3
6α-OCOMe	—		170.5		—		—		—

a) C-24 epimeric mixture.

possessing a 3β,6α-dihydroxy steroid structure.[6] **A** is, thus, considered to have a 24-ethylcholestane-3β,6α-diol structure. Inspection of the ^{13}C- and ^{1}H-NMR signals arising from the side chain of **A** (Tables 1 and 2) and the comparison of their chemical shifts with those of the relevant compounds[7,8] revealed that **A** is not a single compound but a mixture of the C-24 epimers: stigmastane-3β,6α-diol (**1a**; 24*R*) and poriferastane-3β,6α-diol (**1b**; 24*S*) (24*R*:24*S*=*ca.* 1:1). The proposed structure of **1a** was confirmed by its synthesis starting from sitosterol (stigmast-5-ene-3β-ol) acetate (**6a**) (Chart 2). Hydroboration of **6a** followed by treatment with H_2O_2–NaOH[9,10] yielded **1a** accompanied by its 6β-epimer, stigmastane-3β,6β-diol (**7a**) from which **1a** was isolated by reverse phase HPLC. Acetylation of **1a** yielded a diacetate, **2a**. The semi-synthetic **1a** and **2a** were identical by chromatographic and spectral comparison with natural **1a**, a component of **A**, and its diacetate **2a**, respectively.

The peracetate of **B** (**B**-acetate) exhibited the highest mass ion at *m/z* 454.3792 ($M^+ - HOAc$, $C_{31}H_{50}O_2$) in the HR-MS. Alkaline hydrolysis of the acetate yielded a free sterol mixture **B** (*m/z* 430.3820, M^+, $C_{29}H_{50}O_2$). This mixture possesses a saturated 24-ethylated cholestane side chain with two secondary hydroxyl groups and a trisubstituted double bond in the ring system as shown by its ^{13}C- and ^{1}H-NMR data (Tables 1 and 2). One of the hydroxyl groups was suggested to take an equatorial configuration [–CH_2–C<u>H</u>(eq. OH)–CH<], located and oriented most probably at C-3β, from the signal at δ 3.56 (ddd, J=3.6, 4.4, 11.7 Hz) arising from an axial hydroxymethine group, whereas the other hydroxyl group would be axially oriented [>CH–C<u>H</u>(ax. OH)–C(quaternary)–] from the signal at δ 4.14, (br d, J= 3.4 Hz) due to an equatorial hydroxymethine group.[11] The presence of a definite cross-peak between the hydroxymethine signals in the two-dimensional (2D) ^{1}H–^{1}H shift correlated spectroscopy (COSY) revealed that the two hydroxyl groups were located in the vicinity of each other. Furthermore, the 2D nuclear Overhauser effect (NOE) correlation spectroscopy (NOESY) of **B** exhibited a definite NOE correlated peak coupled with the hydroxymethine signal at δ 4.14, and an olefinic signal was observed highly deshielded at δ 5.68 (dd, J=2.1, 5.5 Hz) which suggested the presence of an allylic alcohol system

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Table 2. ^{1}H-NMR Data (δ/ppm) for Three Mixtures of Hydroxylated Sterols **A**—**C** and Their Diacetyl Derivatives from *Trichosanthes kirilowii* Seeds ($CDCl_3$, 400 MHz)[a]

Proton	A[b] Diol		A[b] Diacetate		B[b] Diol		B[b] Diacetate		C Diacetate
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**4c**
3	3.58 (tt, 5.1, 11.0)		4.67 (tt, 4.4, 11.2)		3.56 (ddd, 3.6, 4.4, 11.7)		4.74 (ddd, 3.1, 4.4, 12.5)		4.74 (ddd, 3.3, 4.0, 12.5)
4	—		—		4.14 (d, 2.9)		5.50 (d, 2.6)		5.50 (d, 3.3)
6	3.42 (dt, 4.4, 11.0)		4.67 (dt, 4.4, 11.2)		5.68 (dd, 2.1, 5.5)		5.82 (dd, 2.9, 5.1)		5.81 (dd, 1.8, 4.8)
18	0.65 (s)		0.65 (s)		0.68 (s)		0.67 (s)		0.67 (s)
19	0.82 (s)		0.88 (s)		1.18 (s)		1.13 (s)		1.13 (s)
21	0.91 (d, 5.9)		0.91 (d, 6.8)	0.90 (s, 6.4)	0.92 (d, 6.4)		0.92 (d, 6.2)		0.90 (d, 6.2)
26	0.84 (d, 7.2)	0.83 (d, 7.2)	0.83 (d, 7.2)		0.84 (d, 7.0)	0.83 (d, 7.0)	0.84 (d, 7.3)	0.83 (d, 7.0)	1.57 (s)
27	0.81 (d, 7.2)		0.81 (d, 8.1)		0.81 (d, 6.6)		0.81 (d, 7.7)		4.64 (1H, d, 1.8) 4.73 (1H, br s)
29	0.84 (t, 8.0)	0.85 (t, 7.3)	0.84 (t, 7.8)	0.85 (t, 7.3)	0.85 (t, 7.7)	0.86 (t, 7.3)	0.84 (t, 7.7)	0.85 (t, 7.3)	0.80 (t, 7.7)
3β-OCOMe	—		2.03 (s)		—		2.07 (s)		2.07 (s)
4β-OCOMe	—		—		—		2.01 (s)		2.01 (s)
6α-OCOMe	—		2.03 (s)		—		—		—

a) Figures in parentheses denote *J* values (Hz). *b*) C-24 epimeric mixture.

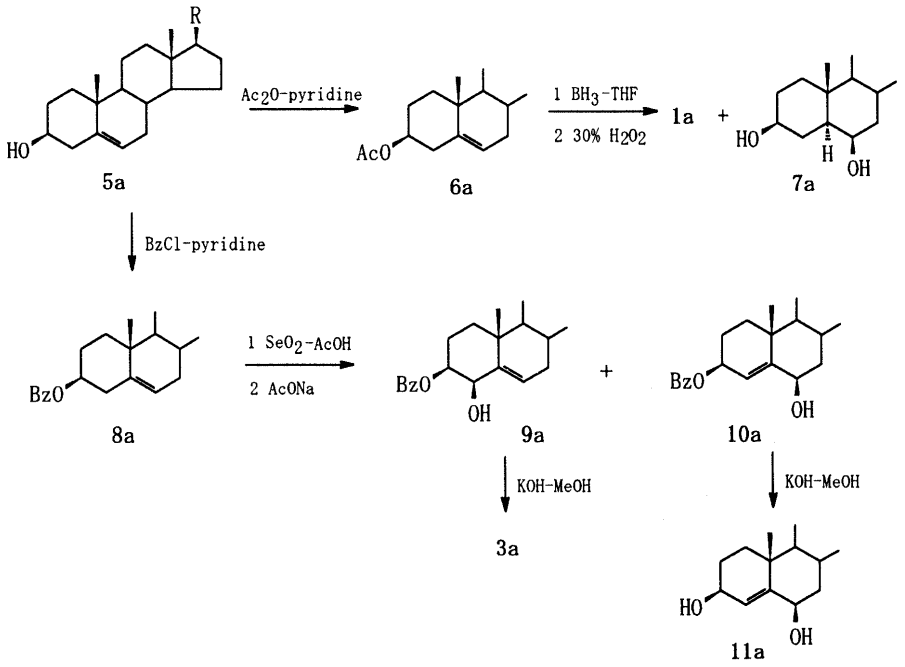

Chart 2. Semi-synthesis of Stigmastane-3β,6α-diol (**1a**) and Stigmast-5-ene-3β,4β-diol (**3a**) from Sitosterol (**5a**)

between the axially oriented hydroxyl group and the trisubstituted double bond. Thus, this had to include the substructure $-CH_2-CH$(eq. OH)$-CH$(ax. OH)$-C$(quaternary)$=CH-CH_2^-$. From the foregoing, **B** was proposed to have a 3β,4β-dihydroxy Δ^5-steroid structure[11-13] and, therefore, to possess the structure 24-ethylcholest-5-ene-3β,4β-diol. The ^{13}C- and ^{1}H-NMR data of **B** and its diacetyl derivative (Tables 1 and 2) revealed that **B** was a mixture of both C-24 epimers: stigmast-5-ene-3β,4β-diol (**3a**; 24*R*) and poriferast-5-ene-3β,4β-diol (**3b**; 24*S*) (24*R*:24*S*=*ca.* 2:3). The proposed structure of **3a** was confirmed by its synthesis starting from sitosteryl benzoate (**8a**) (Chart 2). Treatment of **8a** with selenium dioxide in acetic acid gave 3β-benzoyloxystigmast-5-en-4β-ol (**9a**)[14]

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accompanied by 3β-benzoyloxystigmast-4-en-6β-ol (**10a**) from which **9a** was isolated by reverse phase HPLC. Alkaline hydrolysis of **9a** afforded a diol **3a** which on acetylation yielded a diacetate **4a**. The semi-synthetic **3a** and **4a** were identical according to chromatographic and spectral comparison with natural **3a**, a component of **B**, and its diacetate **4a**, respectively.

The peracetate of **C** (**C**-acetate), which exhibited the M^+ at *m/z* 512.3922 ($C_{33}H_{52}O_4$) in the HR-MS, was the diacetate of a diunsaturated C_{29} hydroxylated sterol as indicated by its ^{13}C- and ^1H-NMR data (Tables 1 and 2). The ^{13}C- (Table 1) and ^1H-NMR (Table 2) signals arising from the ring system of **C**-acetate were indistinguishable from those for the corresponding signals of 3β,4β-diacetoxy Δ^5-steroid (**4a**/**4b**) and, hence, **C** possesses a 3β,4β-dihydroxy Δ^5-steroid structure. The NMR data of **C**-acetate further showed that it possesses a 24-ethyl-Δ^{25}-unsaturated side chain structure.[7,15,16] The ^{13}C- and ^1H-NMR signals due to the side chain of **C**-acetate were indistinguishable from those reported for poriferasta-5,25-dien-3β-yl acetate (24*S*),[16] but were obviously different from those of its 24*R*-epimer, stigmasta-5,25-dien-3β-yl acetate.[16] Thus, **C**-acetate was concluded to be the diacetyl derivative (**4c**) of poriferasta-5,25-diene-3β,4β-diol (**3c**).

Tables 1 and 2 show the ^{13}C- and ^1H-NMR data, respectively, of the hydroxysterols and their acetyl derivatives described above.

Among the five hydroxylated sterols: two 3β,6α-dihydroxy steroids, **1a** and **1b** (isolated as a mixture), and three 3β,4β-dihydroxy Δ^5-steroids, **3a** and **3b** (isolated as a mixture) and **3c**, isolated from the unsaponifiable lipid of *T. kirilowii* seed extract, the latter four are new naturally occurring compounds. Although several compounds possessing a 3β,4β-dihydroxy Δ^5-steroid structure have previously been synthesized,[11-14] this is the first time this type of hydroxylated sterols as the natural products has been detected. Hydroxylated sterol **1a** has previously been isolated from the roots of *Spatholobus suberetus* (Leguminosae)[9] and of *Urtica dioica* (Urticaceae).[17]

Experimental

Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. All hydroxylated sterols and their derivatives were crystallized, if not otherwise specified, as fine needles from acetone–MeOH. Preparative HPLC was carried out on an octadecyl silica column (Altex Ultrasphere ODS 5 μ column, 25 cm × 10 mm i.d.; Beckman Instruments, Inc., San Ramon, California) with MeOH (4 ml/min) using an SSC Flow System 3100K (Senshu Scientific Co., Tokyo) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd., Tokyo). Gas-liquid chromatography (GLC) was run on a Shimadzu GC-14A apparatus using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). In both HPLC and GLC, cholesterol (cholest-5-en-3β-ol) was the standard for the determination of Rt_R(I) of hydroxysterols, whereas cholesteryl acetate was the standard for the determination of Rt_R(II) for the acetyl or benzoyl derivatives of hydroxysterols. Electron-impact (EI) MS and HR-MS were taken on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. NMR spectra were recorded with JEOL GX-400 and GSX-400 spectrometers at 400 MHz (^1H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane (^1H-NMR) and $CDCl_3$ at δ 77.0 (^{13}C-NMR) as internal standards, and chemical shifts were recorded in δ values. Acetylation in Ac_2O–pyridine and benzoylation in benzoyl chloride–pyridine were performed at room temperature overnight. Hydrolysis of acetates and benzoates was done in 5% KOH in MeOH at room temperature overnight. The source of the seed material was described in our recent papers.[2] Sitosterol (**5a**) was isolated from the rhizomes of *Cimicifuga aerina* (Ranunculaceae) in a similar way to that described in the literature.[18]

The signal assignments were performed by comparison with the literature data for the relevant sterols,[7,8,11-13,15,16] and further with the aid of the following NMR experiments: ^{13}C distortionless enhancement by polarization transfer (DEPT), ^1H–^1H COSY, ^1H–^1H NOESY, ^1H–^{13}C COSY, and heteronuclear multiple-bond correlation (HMBC) experiments.[19]

Isolation Procedure Air-dried and ground seeds of *T. kirilowii* (4 kg) were extracted with CH_2Cl_2 in a Soxhlet extractor. Unsaponifiable lipids (17 g) were obtained from the extract (1.2 kg) by alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h) as described previously.[2] The residue (2.13 g) of the most polar of the 9 fractions[2] of silica gel column chromatography was acetylated, and the resultant acetate fraction (1.75 g) was subjected to repeated HPLC, which eventually yielded **A**-acetate (a mixture of **2a** and **2b**; 16 mg), **B**-acetate (a mixture of **4a** and **4b**; 5 mg), and **C**-acetate (**4c**; 8 mg), in addition to several previously described D:C-friedo-oleanane triterpenes,[2,3] and 7-oxo-10α-cucurbita-5,24-dien-3β-ol.[4]

Mixture of Stigmastane-3β,6α-diyl (2a) and Poriferastane-3β,6α-diyl Diacetates (2b) (A-Acetate) Amorphous gum. Rt_R(II): 0.54 (HPLC), 3.20 (GLC). HR-MS *m/z*: 516.4412 [Calcd for $C_{33}H_{56}O_4$ (M^+): 516.4175].

Mixture of Stigmastane-3β,6α-diol (1a) and Poriferastane-3β,6α-diol (1b) (A) mp 214—215 °C. Rt_R(I): 0.50 (HPLC). HR-MS *m/z*: 432.3946 [Calcd for $C_{29}H_{52}O_2$ (M^+): 432.3964]; 249.1830 [Calcd for $C_{16}H_{25}O_2$ (ring D cleavage + 1H): 249.1853]; 231.1756 [Calcd for $C_{16}H_{23}O$ (*m/z* 249 − H_2O): 231.1748]; 213.1661 [Calcd for $C_{16}H_{21}$ (*m/z* 231 − H_2O): 213.1642].

Mixture of Stigmast-5-ene-3β,4β-diyl (4a) and Poriferast-5-ene-3β,4β-diyl Diacetate (4b) (B-Acetate) mp 167—169 °C. Rt_R(II): 0.61 (HPLC), 2.88 (GLC). HR-MS *m/z*: 454.3792 [Calcd for $C_{31}H_{50}O_2$ (M^+ − HOAc): 454.3807].

Mixture of Stigmast-5-ene-3β,4β-diol (3a) and Poriferast-5-ene-3β,4β-diol (3b) (B) mp 174—177 °C. Rt_R(I): 0.21 (HPLC). HR-MS *m/z*: 430.3820 [Calcd for $C_{29}H_{50}O_2$ (M^+): 430.3808].

Poriferasta-5,25-diene-3β,4β-diyl Diacetate (4c) (C-Acetate) mp 165—166 °C. Rt_R(II): 0.46 (HPLC), 2.90 (GLC). HR-MS *m/z*: 512.3922 [Calcd for $C_{33}H_{52}O_4$ (M^+): 512.3863].

Preparation of Stigmastane-3β,6α-diol (1a) and Stigmastane-3β,6β-diol (7a) from Sitosteryl Acetate (6a) **6a** (500 mg, 1.21 mmol) in dry tetrahydrofuran (THF, 7 ml) was treated with 1 M BH_3–THF complex solution (3 ml) at room temperature for 6 h. Then, the reaction mixture was treated with 3 M NaOH (0.5 ml) and 30% H_2O_2 (0.5 ml) at room temperature for 1 h. Work-up using diethyl ether (Et_2O) as a solvent gave a crude product (431 mg). HPLC of the product yielded stigmastane-3β,6α-diol (**1a**) (119 mg) and stigmastane-3β,6β-diol (**7a**) (38 mg).[20] **1a**: mp 217—219 °C (lit.[9] 207—209 °C). Acetylation of **1a** yielded **2a**. Chromatographic and MS data of semi-synthetic **1a** and **2a** were consistent with those of **A** (a mixture of **1a** and **1b**) and **A**-acetate from *T. kirilowii* seed extract, respectively. ^1H-NMR data of semi-synthetic **1a** and **2a** were in accordance with the corresponding compounds from *T. kirilowii* listed in Table 2.

Stigmastane-3β,6β-diol (7a) mp 208—210 °C. Rt_R(I): 0.58 (HPLC). HR-MS *m/z*: 432.3962 [Calcd for $C_{29}H_{52}O_2$ (M^+): 432.3964]. ^1H-NMR δ: 0.69 (3H, s, H-18), 0.81 (3H, d, *J* = 6.9 Hz, H-27), 0.84 (3H, d, *J* = 7.1 Hz, H-26), 0.84 (3H, t, *J* = 7.7 Hz, H-29), 0.91 (3H, d, *J* = 6.6 Hz, H-21), 1.03 (3H, s, H-19), 3.65 (1H, tt, *J* = 5.2, 11.0 Hz, H-3), 3.68 (1H, br d, *J* = 2.8 Hz, H-6). The ^1H signals arising from the ring system of **7a** were indistinguishable from those reported for cholestane-3β,6β-diol,[21] while those due to the side chain were identical with those of **1a** described above, and, thus, **7a** was considered to have the structure stigmastane-3β,6β-diol.

Preparation of Stigmast-5-ene-3β,4β-diol (3a) and Stigmast-4-ene-3β,6β-diol (11a) from Sitosteryl Benzoate (8a) To a suspension of **8a** (500 mg) in acetic acid (5 ml) was added selenium oxide (100 mg) in water (50 μl) and acetic acid (5 ml). The mixture was heated under reflux for 5 min. Sodium acetate (380 mg) was added and the hot solution was filtered through silica gel. Work-up with Et_2O gave a crude product (502 mg) which on preparative HPLC yielded 3β-benzoyloxystigmast-5-en-4β-ol (**9a**) (102 mg) and 3β-benzoyloxystigmast-4-en-6β-ol (**10a**) (71 mg).[22] Alkaline hydrolysis of **9a** gave a diol **3a** (mp 175—178 °C) which on acetylation yielded a diacetate **4a** (mp 167—169 °C). Chromatographic and MS data of semi-synthetic **3a** and **4a** were

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consistent with those of **B** (a mixture of **3a** and **3b**) and **B**-acetate from the seed extract of *T. kirilowii*, respectively. ^{1}H-NMR data of semi-synthetic **3a** and **4a** were indistinguishable from those of the corresponding compounds from *T. kirilowii* described in Table 2. **9a**: mp 195—197 °C. Rt_R(II): 0.63 (HPLC). HR-MS *m/z*: 534.4100 [Calcd for $C_{36}H_{54}O_3$ (M^+): 534.4070]. ^{1}H-NMR δ: 0.69 (3H, s, H-18), 0.82 (3H, d, J=7.0 Hz, H-27), 0.84 (3H, d, J=7.0 Hz, H-26), 0.85 (3H, t, J=8.1 Hz, H-29), 0.93 (3H, d, J=6.6 Hz, H-21), 1.27 (3H, s, H-19), 4.40 (1H, d, J=3.0 Hz, H-4), 4.98 (1H, ddd, J=3.0, 4.4, 12.1 Hz, H-3), 5.75 (1H, dd, J=2.2, 5.5 Hz, H-6), 7.45 (2H, t, J=7.3 Hz, H-Ph), 7.57 (1H, t, J=7.3 Hz, H-Ph), 8.06 (2H, d, J=5.1 Hz, H-Ph).

3β-Benzoyloxystigmast-4-en-6β-diol (10a), Stigmast-4-ene-3β,6β-diol (11a), and Stigmast-4-ene-3β,6β-diyl Diacetate **10a**: Amorphous gum. Rt_R(II): 0.56 (HPLC). HR-MS *m/z*: 534.4033 [Calcd for $C_{36}H_{54}O_3$ (M^+): 534.4069]. ^{1}H-NMR δ: 0.73 (3H, s, H-18), 0.82 (3H, d, J=7.0 Hz, H-27), 0.84 (3H, d, J=7.0 Hz, H-26), 0.85 (3H, t, J=8.1 Hz, H-29), 0.92 (3H, d, J=6.6 Hz, H-21), 1.32 (3H, s, H-19), 4.26 (1H, br s, H-6), 5.50 (1H, ddd, J=1.8, 4.0, 8.4 Hz, H-3), 5.61 (1H, br s, H-4), 7.44 (2H, t, J=7.7 Hz, H-Ph), 7.56 (1H, t, J=7.7 Hz, H-Ph), 8.06 (2H, d, J=7.3 Hz, H-Ph). Alkaline hydrolysis of **10a** yielded a diol **11a**. **11a**: mp 210—213 °C. Rt_R(I): 0.60 (HPLC). HR-MS *m/z*: 430.3803 [Calcd for $C_{29}H_{50}O_2$ (M^+): 430.3807]. ^{1}H-NMR δ: 0.71 (3H, s, H-18), 0.81 (3H, d, J=7.0 Hz, H-27), 0.83 (3H, d, J=7.3 Hz, H-26), 0.84 (3H, t, J=7.7 Hz, H-29), 0.91 (3H, d, J=6.2 Hz, H-21), 1.26 (3H, s, H-19), 4.18 (1H, m, H-3), 4.23 (1H, t, J=2.9 Hz, H-6), 5.54 (1H, br s, H-4). Acetylation of **11a** gave stigmast-4-ene-3β,6β-diyl diacetate: amorphous gum. Rt_R(II): 0.48 (HPLC), 2.72 (GLC). HR-MS *m/z*: 454.3821 [Calcd for $C_{31}H_{50}O_2$ (M^+ − HOAc): 454.3807]. ^{1}H-NMR δ: 0.72 (3H, s, H-18), 0.81 (3H, d, J=7.0 Hz, H-27), 0.84 (3H, d, J=7.3 Hz, H-26), 0.85 (3H, t, J=7.3 Hz, H-29), 0.92 (3H, d, J=6.6 Hz, H-21), 1.17 (3H, s, H-19), 2.03 (3H, s, OAc-3β), 2.06 (3H, s, OAc-6β), 5.24 (1H, ddd, J=1.8, 4.0, 8.4 Hz, H-3), 5.29 (1H, t, J=2.9 Hz, H-6), 5.61 (1H, br s, H-4). The ring system ^{1}H signals of the diacetate were consistent with those reported for cholest-4-ene-3β,6β-diol diacetate,[23] and, therefore, diol **11a** was considered to have the structure stigmast-4-ene-3β,6β-diol.

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19) Assignments for some ^{13}C-NMR signals made on **1a**[9,17] should be revised to be those as shown in Table 1.
20) We obtained **7a** as a by-product through this reaction although it was reported this reaction proceeded stereospecifically to yield **1a**.[9]
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22) We obtained here the allylic-rearrangement by-product, **10a**, in addition to **9a**. Literature described the production of only **9a** by this reaction.[14]
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