[Chem. Pharm. Bull. 32(11)4372—4381(1984)]

Side Chain Structural Requirement for Utilization of Sterols by the Silkworm for Growth and Development. Non-stereoselective Utilization of the 24-Stereoisomeric Pairs of 24-Alkylsterols

YOSHINORI FUJIMOTO, MIKI KIMURA, FATHY A. M. KHALIFA,[1] and NOBUO IKEKAWA*

Department of Chemistry, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152, Japan

(Received March 13, 1984)

Four C-24 epimeric pairs of 24-alkylsterols **1**—**8** were stereoselectively synthesized *via* orthoester Claisen rearrangement of the (22*R*) or (22*S*)-Δ^{23Z} steroid derivatives (**11** and **12**). These compounds were tested on the silkworm larvae, *Bombyx mori*, in order to examine the relationship of the C-24 stereochemical arrangement and utilizability as a nutrient sterol. All of the tested sterols effectively supported the growth and development of the insect and were converted into cholesterol regardless of the C-24 configuration.

Keywords——24-alkylcholesterol; 24-alkyl-22-dehydrocholesterol; orthoester Claisen rearrangement; stereoselective synthesis; insect sterol; silkworm

Insects require a dietary or exogenous source of sterol for normal growth, development and reproduction since they lack the ability for *de novo* sterol biosynthesis. It is well known that phytophagous insects can convert plant sterols such as sitosterol (**1**), campesterol (**5**) and stigmasterol (**3**) into cholesterol (**17**) by removal of the alkyl substituent at C-24. It has been established that the dealkylation pathway of sitosterol involves fucosterol (**18**), fucosterol 24,28-epoxide (**19**) and desmosterol (**20**) (Chart 1).[2] Our detailed studies using the silkworm, *Bombyx mori*, on stereochemical aspects of the intermediate epoxide resulted in the finding

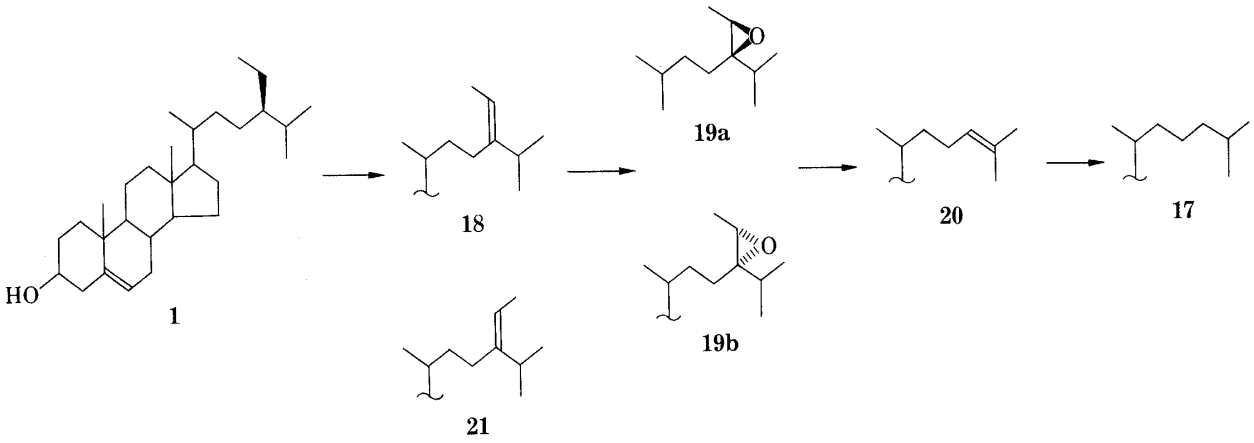


Chart 1. Dealkylation Pathway of Sitosterol in Insects

that formation of the epoxide from fucosterol as well as its conversion into desmosterol proceeded nonstereoselectively; both (24*R*,28*R*)- and (24*S*,28*S*)-fucosterol epoxides (**19a** and **19b**) can be intermediates of sitosterol dealkylation.[3] Subsequently these two epoxides were

identified from silkworm larvae.[4] Furthermore, both fucosterol (**18**) and isofucosterol (**21**) have been recently characterized from the same insect.[5] These facts suggest the lack of rigid stereospecificity in sitosterol dealkylation in *Bombyx mori*, and prompted us to examine in detail whether the silkworm discriminates the C-24 configuration of a variety of 24-alkylsterols available as nutritional sterols. The structures of the four pairs of 24-alkylsterols **1**—**8** employed in our biological studies are illustrated in Chart 2. In this paper we describe

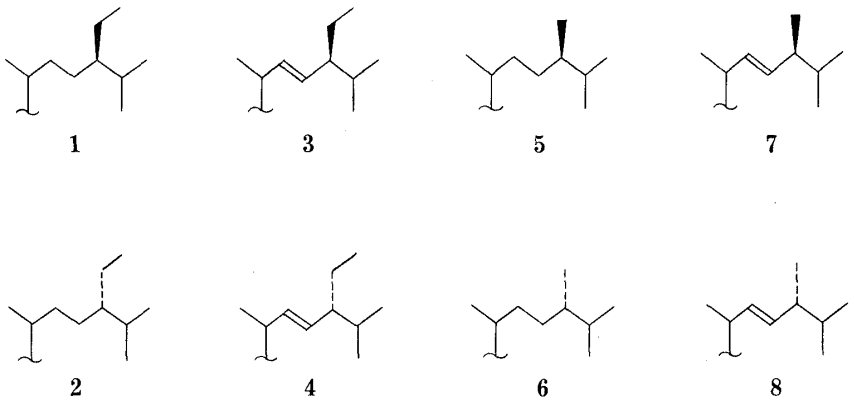


Chart 2. Structure of 24-Alkylsterols

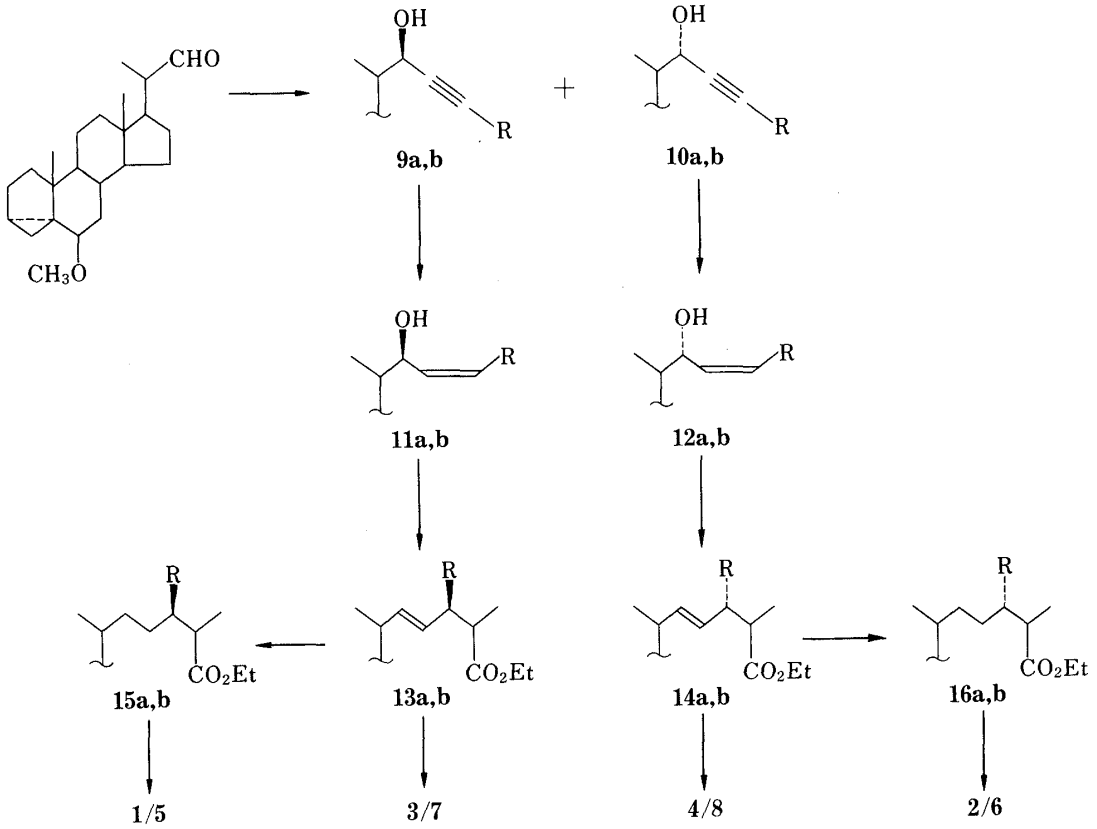
The steroid nucleus is the same as shown in Chart 1.

the synthesis of the sterols **1**—**8** and the results obtained by feeding them to silkworm larvae.

Among several synthetic methods for 24-alkylsterols previously reported,[6] a method involving orthoester Claisen rearrangement of Δ^{23}-22-ol steroids, as exemplified in the synthesis of the oogoniol side chain,[6i,j] appeared to be attractive for our purpose since the methodology has been frequently employed for the construction of 24-substituted sterols and has been established to produce exclusively a single C-24 stereoisomer with a predictable configuration.

Our synthetic route to the 24-alkylsterols **1**—**8** is outlined in Chart 3. In the synthesis of the 24-ethylsterols, there are two possible combinations of the reagents; one involves 3-methyl-1-butyne and ethyl orthoacetate, and the other 1-butyne and ethyl orthopropionate. Sitosterol (**1**) and clionasterol (**2**) have been synthesized by use of the former one.[6i] However, we chose the latter combination since 3-methyl-1-butyne was less accessible and we wanted the intermediate 26-acid (ester) (*vide infra*) for a study of microbial degradation of the phytosterol side chain.[7] The known acetylenic alcohols[6i] **9a** and **10a** were obtained by coupling of the C-22 aldehyde[8] with 1-butynylmagnesium bromide. Compounds **9a** and **10a** were separately hydrogenated over Lindlar catalyst in the presence of quinoline to give the (22*S*)-*cis*-allylic alcohol **11a** and the (22*R*)-epimer **12a**, respectively, in good yields. Compound **11a** was used in the orthoester Claisen rearrangement with triethyl orthopropionate and propionic acid in refluxing xylene to afford the (24*R*)-ester **13a** (82%) as a mixture of C-25 epimers. The (24*R*) stereochemistry was presumed from the generally accepted steric course of this type of rearrangement,[6i,j,9] and it was confirmed by the eventual conversion of **13a** into the natural **1** and **3**. Similarly, the rearrangement of the alcohol **12a** afforded the (24*S*)-ester **14a** (83%). Homogeneity of the C-24 stereochemistry in the esters **13a** and **14a** was established by thin layer chromatography (TLC) analysis; cross contamination was not observed. Thus it is concluded that the orthoester Claisen rearrangement proceeded with high stereoselectivity. Hydrogenation of **13a** and **14a** using 10% Pd/C as a catalyst proceeded smoothly to give the 22-dihydro compounds **15a** and **16a**, in contrast to the finding by Prestwich *et al.*[10]

Further manipulation of the side chain functionality and deprotection of the esters **13a**—**16a** leading to the target sterols **1**—**4** were carried out in a standard manner. Thus, the esters **13a**—**16a** were successively treated, without purification of the intermediates, with $LiAlH_4$ in tetrahydrofuran (THF), methanesulfonyl chloride–pyridine, $LiAlH_4$ in THF, and *p*-toluenesulfonic acid in refluxing aqueous dioxane to give the corresponding 24-ethylsterols, *i.e.* stigmasterol (**3**), poriferasterol (**4**), sitosterol (**1**), and clionasterol (**2**) in yields of approximately 60%.



R=CH_2CH_3 for **a** series
R=CH_3 for **b** series

Chart 3

The 24-methylsterols **5**—**8** were synthesized in the same fashion as the ethyl series. The acetylenic alcohols **9b** and **10b** were prepared by coupling of the C-22 aldehyde with 1-propynylmagnesium bromide according to the literature.[11] Partial hydrogenation of **9b** and **10b** in the same manner as described for **9a** and **10a** quantitatively afforded the (22*S*)- and (22*R*)-*cis*-allylic alcohols[11] **11b** and **12b**, respectively. Orthoester Claisen rearrangement of **11b** and **12b** also proceeded highly stereoselectively to give (24*R*)-ester **13b** and (24*S*)-isomer **14b**, respectively, in good yields. The esters **13b** and **14b**, and their dihydro compounds **15b** and **16b** were transformed into crinosterol (**7**), brassicasterol (**8**), campesterol (**5**), and dihydrobrassicasterol (**6**), respectively. The synthesis of crinosterol using the same methodology has recently been reported.[12]

The structure and homogeneity of the synthetic **1**—**8** were established by melting point determination, and TLC, gas-liquid chromatography (GLC), and proton nuclear magnetic resonance (^{1}H-NMR) analyses. The melting points of **1**—**8** and their acetates, together with the relative retention time (with respect to cholesterol) on GLC and reversed phase

TABLE I. Physical Properties of 24-Alkylsterols and Their Acetates

Alkylsterol	3-Hydroxy form mp (°C)		Rt_R		3-Acetoxy form mp (°C)	
	Observed	Reported	GLC[a]	HPLC[b]	Observed	Reported
1	137.5—139	137—138.5[c] 136.5—138[d]	1.62	1.22	121—122	121.5—122.5[c] 120.5—121.5[e]
2	141—143	141—142.5[d] 139—140[c]	1.62	1.22	141.5—142.5	142—142.5[c] 140—141[f]
3	168—170	169—170[c,g]	1.44	1.10	144—145	144—145[c]
4	156—157	156—157[g] 156—157.5[c]	1.44	1.08	147—148	146—147.5[c]
5	157.5—159.5	160—161[d] 156—158[h]	1.30	1.11	137.5—139.5	139—141[h]
6	158—160	158.5—160[d] 158—159[j]	1.30	1.11	144—146	145[i] 146—148[j]
7	155—157	152—154[k] 147—148[l]	1.14	0.90	151—152	157—158[l]
8	145—147	148[m]	1.14	0.93	150—152	158[m]

a) 1% OV-17, column temperature = 270 °C, cholesterol = 1.00 (4.2 min).
b) Zorbax ODS, eluent = methanol, cholesterol = 1.00 (20 min).
c) Ref. 6*i*. d) Ref. 6*k*. e) Ref. 24. f) Ref. 25. g) Ref. 26. h) Ref. 6*d*.
i) Ref. 27. j) Ref. 6*b*. k) Ref. 12. l) Ref. 14. m) Ref. 28.

TABLE II. Effect of Sterols on Growth and Development of the Silkworm[a]

Sterol	After 10 d			After 17 d		
	Av. wt. (mg)	No. of larvae of the instar		Av. wt. (mg)	No. of larvae of the instar	
		1st	2nd		2nd	3rd
1	18.8	0	20	37.6	0	20
2	17.4	0	20	41.6	0	20
3	21.0	0	20	44.0	0	20
4	17.2	0	20	36.0	0	20
5	19.6	0	20	30.6	0	20
6	19.0	0	20	35.4	0	20
7	15.0	0	20	34.2	6	14
8	14.6	3	17	28.0	8	12
Cholesterol	16.6	0	20	32.6	5	15
Not added	All dead					

a) Twenty larvae were reared on artificial diet including the specified sterol.

high-performance liquid chromatography (HPLC), are listed in Table I. Neither TLC nor GLC could differentiate the C-24 isomers (**1** *vs.* **2**; **3** *vs.* **4**; **5** *vs.* **6**; **7** *vs.* **8**), but ^{1}H-NMR spectroscopy could, as previously reported.[13] The doublet due to the 20-methyl group of **7** appeared at a slightly higher field than that of the 24-epimer **8** in agreement with the previous findings.[14] As noted by Djerassi *et al.*,[15] reversed phase HPLC resolved the 24-stereoisomers having the (22*E*)-double bond, a better separation being obtained with the 24-methylsterols (**7** *vs.* **8**) than the 24-ethylsterols (**3** *vs.* **4**).

The required 24-alkylsterols **1**—**8** being in hand, these compounds were next tested for

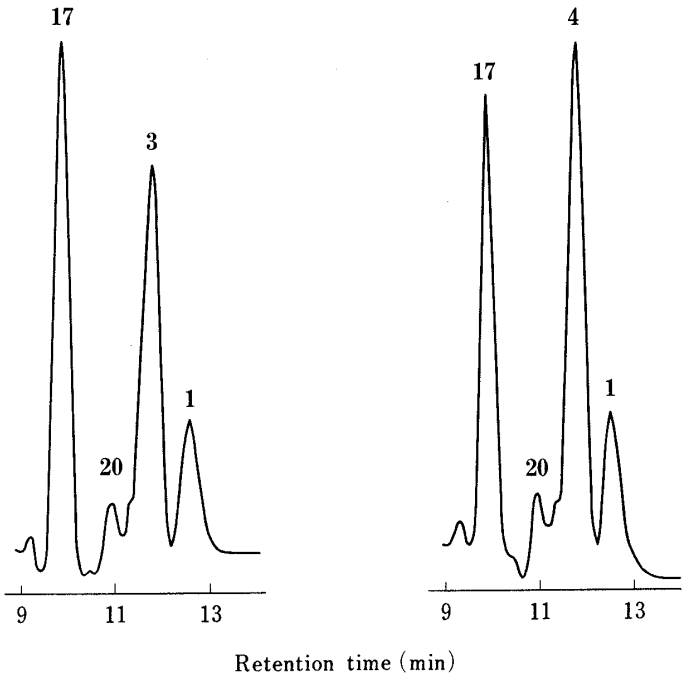


Fig. 1. Gas Chromatogram of the Insect Sterols (TMS ether)
Left: fed with **3**. Right: fed with **4**.

TABLE III. The Ratio of Cholesterol to Fed Sterol

Fed sterol	Cholesterol/fed sterol[a)]
1	48/52
2	50/50
3	56/44
4	45/55
5	42/58
6	35/65
7	44/56
8	39/61

a) In all runs, small amounts of desmosterol and sitosterol were detected, and these were subtracted before calculating the above ratios.

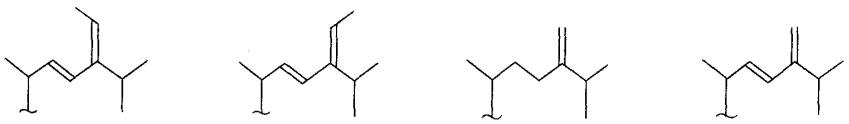
ability to sustain the growth of the silkworm. Newly hatched larvae were fed with artificial diet containing the 24-alkylsterols **1**—**8** (0.1%). The growth and development of the larvae are summarized in Table II. It can be seen that most of the larvae developed to the third instar within 17 d and their mean body weights were at the same level as observed for the control (insects fed cholesterol). According to our classification,[16)] sterols **1**—**8** were all classified as effective sterols for silkworm growth. More importantly, no significant difference between any of the epimeric pairs, *e.g.* **1** *vs.* **2**, as a nutritional sterol could be observed, suggesting that the silkworm can dealkylatively metabolize them into cholesterol irrespective of the C-24 stereochemistry. This was substantiated by gas chromatography-mass spectrometry (GC-MS) analysis of the insect sterol. As an example, the GLC profile of the insect sterol (insects fed with **3** and **4**) is illustrated in Fig. 1. It can be seen that cholesterol apparently produced by dealkylation of the dietary sterol is the major sterol component; the dietary sterol **3** or **4** (M^+ 484) and small amounts of desmosterol (**20**) (M^+ 456) and sitosterol[17)] (**1**) (M^+ 476) were also

detected. The other sterols tested afforded similar results. The ratio of cholesterol to dietary sterol is summarized in Table III. GLC analysis of the saponifiable fraction indicated comparable sterol distributions (data not shown). Thus, it can now be concluded that the silkworm larvae are able to convert both C-24-epimers of 24-alkylsterols **1**—**8** into cholesterol without regard for the stereochemistry at the C-24 position. The looseness of the stereochemical requirement at the C-24 position is in contrast to the rigidity at C-20; only the natural isomer (20*R*) supports silkworm growth.[18]

To our knowledge, three papers have been published on the utilization of C-24 stereoisomers in insects. Low specificity in the utilization of 24-methylcholesterols **5** and **6** was demonstrated with *Manduca sexta*.[19] The larvae of *Dermestes macultus* differentially utilize campesterol acetate (**5**-acetate) and dihydrobrassicasterol acetate (**6**-acetate), with the former being preferred. This insect, however, appeared to have no ability to utilize sitosterol acetate (**1**-acetate) or clionasterol acetate (**2**-acetate).[6e] As regards *Drosophila* species, *D. melanogaster* utilizes both (24*R*)-and (24*S*)-methylcholesta-5,7-dien-3β-ol, while *D. pachea* prefers the 24*S*-isomer and *D. mojavensis* prefers the 24*R*-isomer.[20]

In the case of the silkworm, the easy adaptation to a variety of less common alkylsterols such as **2**, **4**, **6** and **7** is rather surprising, but could be explained by assuming either that the substrate specificity of the enzyme is low or that the silkworm can induce enzyme systems specific for the 24*R* and 24*S* isomers. The answer to this problem must await further investigation.

Some information on the immediate metabolites of the 24-alkylsterols dealkylation is available. We have already reported that fucosterol (**18**) and isofucosterol (**21**) satisfy the sterol requirement of the silkworm.[16] Similarly, the 22,24(28)*Z*-diene (**22**) and 22,24(28)*E*-diene (**23**) also sustain the growth and development of the silkworm.[21] The 22,24(28)-diene



22 23 24 25

(**25**)[22] and 24-methylenecholesterol (**24**)[16] were shown to support silkworm growth effectively. Therefore the first enzymic reaction common to all of the tested 24-alkylsterols should be the introduction of the double bond leading to $\Delta^{24(28)}$-intermediates, which are then epoxidized, rearranged to desmosterol, and reduced to cholesterol. Results which support the intermediacy of the 24,28-epoxides in the dealkylation of campesterol and stigmasterol have recently been published.[21,23] The detection of desmosterol in the insect sterols in all the runs also supports this pathway. Our current idea is that a single enzyme may be responsible for the dehydrogenation of all the substrates **1**—**8**.

Experimental

Melting points were determined on a Yazawa hot stage microscope and are uncorrected. ^{1}H-NMR spectra were recorded on a Hitachi R-24A (60 MHz) (unless otherwise noted), JEOL JNH-PS-100 (100 MHz), or Nicolet NT-360 (360 MHz) spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal reference. In the ^{1}H-NMR of 6β-methoxy-3α,5-cyclo form, data for cyclopropane protones (δ: 0.3—0.7, m, 4-H_2) are not given. *Rf* values reported were obtained on Merck Kieselgel 60 F254 precoated plates (0.25 mm thickness). GLC was performed on a Shimadzu GC-4BM instrument equipped with a 1.5 m × 3 mm i.d. glass column containing 1% OV-17 on Shimalite W, operating at 270 °C, and the retention time was reported as a relative value Rt_R with respect to cholesterol. HPLC was performed with a Shimadzu LC-4A instrument equipped with a ultraviolet (UV) detector (215 nm) using a Zorbax ODS reversed phase column (4.6 mm × 25 cm) and commercial methanol as an eluent at a flow speed of 1.0 ml/min.

MS were obtained with a Shimadzu LKB 9000S spectrometer operating in a GC-MS mode (accelerating voltage, 20 eV). High resolution mass spectra were obtained with a Hitachi M-80 spectrometer. The 3β-acetates of alkylaterol **1**—**8** were prepared in a usual manner (Ac_2O–pyridine) and crystallized from methanol.

(22*S*,23*Z*)-6β-Methoxy-26-nor-3α,5-cyclo-5α-cholest-23-en-22-ol(11a)——A mixture of the (22*R*)-acetylenic alcohol **9a**[6i] (1.30 g, 3.27 mmol), Lindlar catalyst (264 mg, Kawaken Fine Chemicals) and quinoline (1.3 ml) in AcOEt (200 ml) was stirred under a hydrogen atmosphere for 3 h. The mixture was washed with 2 N HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to leave **11a** (1.30 g, 99%) as a solid. Recrystallization from AcOEt afforded an analytical sample, mp 136.5—137.5 °C. ^{1}H-NMR (100 MHz) δ: 0.72 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.76 (1H, m, 6-H), 3.33 (3H, s, OMe), 4.46 (1H, br d, J=5.5 Hz, 22-H), 5.47 (2H, m, 23-H and 24-H). GLC (Rt_R): 0.84. *Rf* value in TLC (hexane : AcOEt=6 : 1, developed twice): 0.45. MS *m/z*: 400 (M^+), 368, 345, 316, 315, 301, 284, 283, 255, 253. *Anal.* Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80, 72; H, 11.09.

(22*R*,23*Z*)-6β-Methoxy-26-nor-3α,5-cyclo-5α-cholest-23-en-22-ol (12a)——Hydrogenation of **10a**[6i] (1.70 g, 4.27 mmol) in the same manner as described for **9a** afforded **12a** (1.52 g, 89%) as a solid. Crystallization from AcOEt afforded an analytical sample, mp 112—113 °C. ^{1}H-NMR (100 MHz) δ: 0.75 (3H, s, 18-Me), 1.00 (3H, d, J=6 Hz, 21-Me), 1.02 (3H, s, 19-Me), 2.2 (2H, q, J=7 Hz, 25-H_2), 2.73 (1H, m, 6-H), 3.31 (3H, s, OMe), 4.44 (1H, dd, J=8 and 4 Hz, 22-H), 5.5 (2H, m, 23-H and 24-H). GLC (Rt_R): 0.84. *Rf* value in TLC (hexane : AcOEt=6 : 1, developed twice): 0.29. *Anal.* Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.26; H, 10.78.

Ethyl (22*E*,24*R*)-6β-Methoxy-3α,5-cyclo-5α-stigmast-22-en-26-oate (13a)[6i]——A solution of **11a** (1.50 g, 3.75 mmol), triethyl orthopropionate (3.75 ml, 18.7 mmol) and propionic acid (0.179 ml, 2.40 mmol) in xylene (75 ml) was heated at reflux under argon. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (80 g). Elution with hexane : AcOEt (20 : 1) gave **13a** (1.5 g, 83%) as an oil. The ^{1}H-NMR and MS were in good agreement with those reported.[6i] GLC (Rt_R): 1.92. *Rf* value in TLC (hexane : AcOEt=15 : 1, developed twice): 0.42.

Ethyl (22*E*,24*S*)-6β-Methoxy-3α,5-cyclo-5α-stigmast-22-en-26-oate (14a)[6i]——Claisen rearrangement of **12a** (1.52 g, 3.8 mmol) in the same way as described for **11a** afforded **14a** (1.52 g, 83%) as an oil, which solidified upon standing, mp 61—63 °C (this material was reported as an oil).[6i] ^{1}H-NMR and MS were in good agreement with those reported.[6i] GLC (Rt_R): 1.87. *Rf* value in TLC (hexane : AcOEt=15 : 1, developed twice): 0.47.

Ethyl (24*R*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-26-oate (15a)——A mixture of **13a** (310 mg, 0.640 mmol) and 10% Pd/C (35 mg) in AcOEt (16 ml) was stirred under a hydrogen atmosphere at room temperature overnight. The catalyst was filtered off through a silica gel pad and the filtrate was evaporated *in vacuo* to give **15a** (310 mg, 100%) as an oil. ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.23 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.72 (1H, m, 6-H), 3.30 (3H, s, OMe), 4.10 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$). GLC (Rt_R): 2.12. *Rf* value in TLC (hexane : AcOEt=15 : 1, developed twice): 0.45. MS *m/z*: 486 (M^+), 471, 454 (base peak), 431, 333, 255. High resolution MS: Calcd for $C_{32}H_{54}O_3$: 486.4070. Found: 486.4065.

Ethyl (24*S*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-26-oate (16a)——The ester **14a** (310 mg, 0.640 mmol) was hydrogenated in the same manner as described for **13a** to give **16a** (310 mg, 99%) as an oil. ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.22 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.73 (1H, m, 6-H), 3.30 (3H, s, OMe), 4.09 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$). GLC (Rt_R): 2.10. *Rf* value in TLC (hexane : AcOEt=15 : 1, developed twice): 0.46. The MS was essentially identical with that of **15a**. High resolution MS: Calcd for $C_{32}H_{54}O_3$: 486.4070. Found: 486.4068.

Conversion of the Ester 13a into Stigmasterol (3)——The ester **13a** (287 mg, 0.593 mmol) in THF (6 ml) was reduced with $LiAlH_4$ (44 mg, 1.16 mmol). The mixture was stirred for 1 h at room temperature under argon, then moist ether and finally 2 N HCl were added. Extractive (ether) work-up gave the 26-ol (256 mg, 97%) as an oil. ^{1}H-NMR δ: 0.74 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 3.30 (3H, s, OMe), 3.42 (2H, d, J=6 Hz, 26-H_2), 5.14 (2H, m, 22-H and 23-H). GLC (Rt_R): 1.76. *Rf* value in TLC (hexane : AcOEt=15 : 1, developed twice): 0.22. MS *m/z*: 442 (M^+), 427, 410, 395, 387, 255, 253. High resolution MS: Calcd for $C_{30}H_{50}O_2$: 442.3808. Found: 442.3823.

A solution of the 26-ol (256 mg, 0.576 mmol) and methanesulfonyl chloride (0.17 ml, 2.15 mmol) in pyridine (2.6 ml) was stirred at room temperature for 2 h. The usual work-up (extraction with ether) gave the crude mesylate (277 mg). ^{1}H-NMR δ: 0.72 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 2.96 (3H, s, SMe), 3.31 (3H, s, OMe), 4.0 (2H, m, 26-H_2), 5.13 (2H, 22-H and 23-H).

$LiAlH_4$ (166 mg, 4.73 mmol) was added to the mesylate (277 mg) in THF (8.3 ml) in several portions and the mixture was stirred at room temperature for 2 h. Extractive (ether) work-up gave the *i*-stigmasterol (187 mg) as an oil. The ^{1}H-NMR spectrum was in good agreement with that reported.[6i] GLC (Rt_R): 0.63. MS *m/z*: 426 (M^+), 411, 394, 371, 341, 255 (base peak), 253, 83.

A mixture of the *i*-stigmasterol (187 mg) and a catalytic amount of *p*-TsOH · H_2O in dioxane (4 ml)–water (1.6 ml) was refluxed for 3 h. Extractive (ether) work-up gave the crude product, which was chromatographed on silica gel (15 g). Elution with hexane–AcOEt (5 : 1) afforded **3** (150 mg, 62% from **14a**) as white crystals. Crystallization from methanol furnished 104 mg (first crop), mp 168—170 °C. All physicochemical properties were identical with those reported.[6i,13]

Conversion of the Ester 14a into Poriferasterol (4)——The ester **14a** (285 mg) was treated in the manner described above. Some properties of the intermediates are listed below.

The 26-ol: ^{1}H-NMR δ: 0.73 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 3.30 (3H, s, OMe). 3.44 (2H, d, J=6 Hz, 26-H_2), 5.16 (2H, m, 22-H and 23-H). GLC (Rt_R): 1.76. *Rf* value in TLC (hexane : AcOEt=15:1, developed twice): 0.27. High resolution MS: Calcd for $C_{30}H_{50}O_2$: 442.3808. Found: 442.3825.

The Mesylate: ^{1}H-NMR δ: 0.72 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 2.96 (3H, s, SMe), 3.31 (3H, s, OMe), 4.0 (2H, m, 26-H_2), 5.13 (2H, m, 22-H and 23-H).

i-Poriferasterol: GLC (Rt_R), 0.63. The ^{1}H-NMR spectrum was identical with that reported[6i] and the MS was the same as for the corresponding 24-isomer described above.

A yield of 185 mg (76% from **14a**) of crystalline **4** was obtained after chromatography. Recrystallization from methanol afforded an analytical sample, mp 156—157 °C. All physico-chemical properties were identical with those reported.[6i,13]

Conversion of the Ester 15a into Sitosterol (1)——The ester **15a** (310 mg) was converted into **1** as described above.

The 26-ol: ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.74 (1H, m, 6-H), 3.30 (3H, s, OMe), 3.5 (2H, m, 26-H_2). GLC (Rt_R): 2.05. *Rf* value in TLC (hexane : AcOEt=15:1, developed twice): 0.22. MS *m/z*: 444 (M^+), 429, 426, 412, 394, 389, 255 (base peak). High resolution MS: Calcd for $C_{30}H_{52}O_2$: 444.3965. Found: 444.3972.

The Mesylate: ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 2.74 (1H, m, 6-H), 2.99 (3H, s, SMe), 3.30 (3H, s, OMe), 4.10 (2H, m, 26-H_2).

i-Sitosterol: ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.74 (1H, m, 6-H), 3.30 (3H, s, OMe), GLC (Rt_R): 0.70. MS *m/z*: 428 (M^+), 413, 396 (base peak), 373, 255. High resolution MS: Calcd for $C_{30}H_{52}O$: 428.4015. Found: 428.4011.

A yield of 140 mg (53% from **15a**) of crystalline **1** was obtained. An analytical sample was obtained by recrystallization from methanol, mp 137.5—139 °C. All physico-chemical properties were identical with those reported.[6k,13] ^{1}H-NMR (360 MHz) δ: 0.680 (3H, s, 18-Me), 0.814 (3H, d, J=7.7 Hz, 26-Me), 0.835 (3H, d, J=7.5 Hz, 27-Me), 0.845 (3H, t, J=7.5 Hz, 29-Me), 0.922 (3H, d, J=6.6 Hz, 21-Me), 1.009 (3H, s, 19-Me), 3.5 (1H, m, 3-H), 5.36 (1H, m, 6-H).

Conversion of the Ester 16a into Clionasterol (2)——The ester **16a** (310 mg) was converted into **2** in the same manner as described above.

The 26-ol: ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.74 (1H, m, 6-H), 3.31 (3H, s, OMe), 3.5 (2H, m, 26-H_2). GLC (Rt_R): 2.06. *Rf* value in TLC (hexane : AcOEt=15:1, developed twice): 0.26. High resolution MS: Calcd for $C_{30}H_{52}O_2$: 444.3965. Found: 444.3983.

The Mesylate: ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 2.99 (3H, s, SMe), 3.30 (3H, s, OMe), 4.09 (2H, m, 26-H_2).

The *i*-Clionasterol: ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 2.73 (1H, m, 6-H), 3.30 (3H, s, OMe). GLC (Rt_R): 0.70. High resolution MS: Calcd for $C_{30}H_{52}O$: 428.4105. Found: 428.4010.

A yield of 165 mg (62% from **16a**) of crystalline **2** was obtained. Recrystallization from methanol afforded an analytical sample, mp 141—143 °C. All physico-chemical properties were identical with those reported.[6k,13] ^{1}H-NMR (360 MHz) δ: 0.680 (3H, s, 18-Me), 0.812 (3H, d, J=7.0 Hz, 26-Me), 0.832 (3H, d, J=7.0 Hz, 27-Me), 0.855 (3H, t, J=7.4 Hz, 29-Me), 0.926 (3H, d, J=6.5 Hz, 21-Me), 1.009 (3H, s, 19-Me), 3.53 (1H, m, 3-H), 5.36 (1H, m, 6-H).

(22*S*,23*Z*)-6β-Methoxy-26,27-bisnor-3α,5-cyclo-5α-cholest-23-en-22-ol (11b)[11]——The acetylenic alcohol **9b** (1.50 g), which was obtained according to the procedure of Hirano and Djerassi,[11] was hydrogenated in the same manner as described for **9a**, affording **11b** (1.50 g, 98%) as a solid. An analytical sample was obtained by crystallization from hexane–AcOEt, mp 137—139 °C (lit.[11] 85—87 °C). GLC (Rt_R): 0.73. *Rf* value in TLC (hexane : AcOEt=6:1, developed twice): 0.44. The ^{1}H-NMR spectrum was identical with that reported.[11]

(22*R*,23*Z*)-6β-Methoxy-26,27-bisnor-3α,5-cyclo-5α-cholest-23-en-22-ol (12b)[11]——Hydrogenation of **10b**[11] (1.50 g) in the same manner as described above gave **12b** (1.5 g, 98%) as an amorphous solid. GLC (Rt_R): 0.76. *Rf* value in TLC (hexane : AcOEt=6:1, developed twice): 0.40. The ^{1}H-NMR spectrum was identical with that reported.[11]

Ethyl (22*E*,24*R*)-6β-Methoxy-3α,5-cyclo-5α-ergost-22-en-26-oate (13b)——Orthoester Claisen rearrangement of **11b** (2.40 g) in the same manner as described for **11a** afforded **13b** (2.40 g, 82%) as an oil. ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.23 (3H, t, J=8 Hz, OCH_2CH_3), 2.73 (1H, m, 6-H), 3.31 (3H, s, OMe), 4.11 (2H, q, J=8 Hz, $OC\underline{H}_2CH_3$), 5.2 (2H, m, 22-H and 23-H). GLC (Rt_R): 1.55. *Rf* value in TLC (hexane : AcOEt=20:1, developed twice): 0.36. MS *m/z*: 470 (M^+), 455, 438, 423, 415, 255 (base peak), 253, 183. High resolution MS: Calcd for $C_{31}H_{50}O_3$: 470.3757. Found: 470.3742.

Ethyl (22*E*,24*S*)-6β-Methoxy-3α,5-cyclo-5α-ergost-22-en-26-oate (14b)——Treatment of **12b** (1.50 g) in the same manner as described for **11a** afforded **14b** (1.50 g, 82%) as crystals, mp 100—102 °C (methanol). ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.25 (3H, t, J=8 Hz, $OCH_2C\underline{H}_3$), 2.77 (1H, m, 6-H), 3.31 (3H, s, OMe), 4.16 (2H, q, J=8 Hz, $OC\underline{H}_2CH_3$), 5.25 (2H, m, 22-H and 23-H). GLC (Rt_R): 1.60. *Rf* value in TLC (hexane : AcOEt=20:1, developed twice): 0.40. The MS was the same as for **13b**. *Anal.* Calcd for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.38; H, 10.84.

Ethyl (24*R*)-6β-Methoxy-3α,5-cyclo-5α-ergostan-26-oate (15b)——Hydrogenation of **13b** (300 mg) in the same

manner as described for **13a** afforded **15b** (290 mg, 96%) as an oil. ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.23 (3H, t, J=7 Hz, OCH_2CH_3), 2.72 (1H, m, 6-H), 3.30 (3H, s, OMe), 4.10 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$). GLC (Rt_R): 1.81. MS m/z: 472 (M^+), 457, 440, 417, 319, 283, 255, 213. High resolution MS: Calcd for $C_{31}H_{52}O_3$: 472.3914. Found: 472.3928.

Ethyl (24*S*)-6β-Methoxy-3α,5-cyclo-5α-ergostan-26-oate (16b)——Hydrogenation of **14b** (300 mg) in the same manner as described for **11a** afforded **16b** (285 mg, 95%). ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.22 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.73 (1H, m, 6-H), 3.30 (3H, s, OMe), 4.09 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$). GLC (Rt_R): 1.94. The MS was the same as for **15b**. High resolution MS: Calcd for $C_{31}H_{52}O_3$: 472.3914. Found: 472.3922.

Conversion of the Ester 13b into Crinosterol (7)——When the ester **13b** (300 mg) was treated in the same way as described for **13a**, 180 mg (71%) of crystalline **7** was obtained, mp 155—157 °C (methanol). ^{1}H-NMR (360 MHz) δ: 0.695 (3H, s, 18-Me), 0.819 (3H, d, J=6.5 Hz, 28-Me), 1.003 (3H, d, J=6.5 Hz, 21-Me), 1.011 (3H, s, 19-Me), 3.5 (1H, m, 3-H), 5.17 (2H, m, 22-H and 23-H), 5.35 (1H, m, 6-H). It was confirmed that the sample was free from the 24-epimer **8** by HPLC analysis (see Table I).

The intermediates showed the following properties. The 26-ol: ^{1}H-NMR δ: 0.72 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.75 (1H, m, 6-H), 3.31 (3H, s, OMe), 3.48 (2H, m, 26-H_2), 5.21 (2H, m, 22-H and 23-H). *Rf* value (hexane : AcOEt = 4 : 1, developed twice): 0.26. GLC (Rt_R): 1.70. MS m/z: 428 (M^+), 413, 396, 373, 255 (base peak), 253. High resolution MS: Calcd for $C_{29}H_{48}O_2$: 428.3651. Found: 428.3621. *i*-Crinosterol: ^{1}H-NMR δ: 0.72 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 2.75 (1H, m, 6-H), 3.31 (3H, s, OMe), 5.18 (2H, m, 22-H and 23-H). GLC (Rt_R): 0.52. MS m/z: 412 (M^+), 397, 380, 365, 357, 255 (base peak).

Conversion of the Ester 14b into Brassicasterol (8)——The ester **14b** (300 mg) was similarly converted into 175 mg (69%) of crystalline **8**, mp 145—147 °C. ^{1}H-NMR (360 MHz) δ: 0.694 (3H, s, 18-Me), 0.820 (3H, d, J=6.7 Hz, 26-Me), 0.836 (3H, d, J=6.7 Hz, 27-Me), 0.912 (3H, d, J=6.9 Hz, 28-Me), 1.011 (3H, s, 19-Me), 1.013 (3H, d, J=6.6 Hz, 21-Me), 3.5 (1H, m, 3-H), 5.18 (2H, m, 22-H and 23-H), 5.35 (1H, m, 6-H).

The intermediates, the 26-ol and *i*-brassicasterol, had essentially the same properties as the corresponding C-24 isomers described above except for the *Rf* value (identical system) of the 26-ol: 0.28.

Conversion of the Ester 15b into Campesterol (5)——The ester **15b** (300 mg) was similarly converted into 145 mg (57%) of crystalline **5**, mp 157.5—159.5 °C (methanol). All physico-chemical properties including the ^{1}H-NMR spectrum (100 MHz) were identical with those reported.[6k,13]

The intermediates, the 26-ol and *i*-campesterol, showed GLC (Rt_R): 1.70 and 0.58, and MS m/z: 430 (M^+), 415, 398, 375, 255 and 414 (M^+), 399, 382, 359, 255, respectively.

Conversion of the Ester 16b into Dihydrobrassicasterol (2)——The ester **16b** (285 mg) was similarly converted into 141 mg (59%) of crystalline **2**, mp 158—160 °C (methanol). All physico-chemical properties including the ^{1}H-NMR spectrum (100 MHz) were identical with those reported.[6k,13]

Biological Test——Artificial diet containing 0.1% (wet wt.) of the test sterol was prepared, and newly hatched *Bombyx mori* larvae (20 specimens in each group) were reared as described previously.[16] On day 17 after hatching, the insects (mostly 3rd instar) were ground with silica gel/$CHCl_3$–methanol (2 : 1) in a mortar. The whole mixture was filtered through cotton and the filtrate was concentrated. An aliquot was treated with *N*-trimethylsilylimidazole and the resulting TMS–ether(s) was analyzed by GLC (Shimadzu GC-7A; 1% OV-17 on Shimalite W, 1.5 m × 3 mm i.d.; gradient temperature from 220 to 280 °C at 4 °C/min) and/or GC-MS (Shimadzu LKB 9000S, essentially the same conditions as above). In some instances, another aliquot was saponified with 5% KOH–methanol (reflux, 1 h) and the non-saponifiable fraction (AcOEt extract) was analyzed similarly.

Acknowledgement The authors wish to thank Drs. T. Iwashita, Suntory Institute for Bioorganic Research, and M. Ishiguro, Suntory Institute for Biomedical Research, for 360 MHz ^{1}H-NMR measurement. We also thank Dr. M. Morisaki of this institute for valuable advice during this study. Finally, we thank Japan Upjohn Ltd. for a generous supply of stigmasterol.

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