Minor and Trace Sterols from Marine Invertebrates. 50.[1] Stereostructure and Synthesis of Nicasterol, a Novel Cyclopropane-Containing Sponge Sterol

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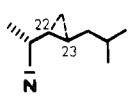
The isolation, structure elucidation, and partial synthesis of nicasterol, the first naturally occurring sterol of the 23,25-cyclocholesterol type, is described.

The occurrence of a cyclopropane ring in the side chain constitutes a structural feature unique to marine sterols. The structural types encountered so far range from simple[3] (1) and more substituted[4,5] (e.g., 2 and 3) 22,23-methylenecholesterols to examples in which the cyclopropane ring arises by bond formation between C24 and C26[6] (4), C23 and C28[7a,b,c] (5) and finally C26 and C27[8] (6). We now describe still another cyclopropane type—arising from C23–C25 bond formation—which was encountered among the sterols of the sponge *Calyx nicaensis* already known as a rich source of cyclopropanes[7b] and cyclopropenes[7a–c] derived from the basic structure 5.



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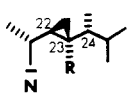
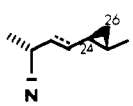


2 R = Me
3 R = H



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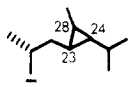
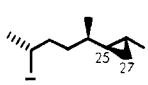
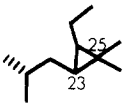




5a 28R, Δ23,24
b 23R, Δ24,28
c 24S, Δ23,28
d 23S,24S,28R

6

Fractionation of the sterol mixture of *Calyx nicaensis* by reverse-phase HPLC (Altex Ultrasphere ODS-5, mobile phase methanol–water 98/2) provided a new trace sterol, for which we suggest the trivial name, nicasterol. Nicasterol showed a molecular ion at m/z 412.3727 ($C_{29}H_{48}O$ requires 412.3705) and a fragmentation pattern typical of the cholesterol nucleus[9] (fragment ions at m/z 215.1798, $C_{16}H_{23}$; 229.1596, $C_{16}H_{21}O$; 255.2097, $C_{19}H_{27}$; 271.2071, $C_{19}H_{27}O$). A diagnostic fragmentation peak at m/z 314.2607 ($M - C_7H_{14}$) suggested the presence of unsaturation around C24 in the side chain.[10] That this unsaturation was due to a cyclopropane ring was demonstrated by the 360-MHz ^1H NMR spectrum (Table I) which also pointed to the presence of an ethyl group and two quaternary methyl groups in addition to the C18, C19, and C21 methyl groups. These data along with basic biosynthetic considerations[11] led to the formulation of nicasterol as 23,25-cyclostigmast-5-en-3β-ol (7) of undetermined stereochemistry around the cyclopropane ring. In order to confirm this structure and acquire sufficient material to allow assignment of the absolute stereochemistry the following synthesis of nicasterol was undertaken.



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The tosylate 8, available in four steps from stigmasterol[12] was converted, via the nitrile 9, to the aldehyde 10. A Wittig reaction with isopropylidenetriphenylphosphorane yielded olefin 11 which was subjected to a carbene addition with ethyl diazoacetate to give a mixture of four diastereomeric cyclopropanecarboxylic acid esters **12a–d**. Two of the esters could be separated in pure form by reverse-phase HPLC, and the remaining two isomers were carried forward to the next reaction as a mixture. Reduction of the esters **12a–d** with lithium aluminum hydride gave the four alcohols **13a–d**, all of which could be separated and purified by reverse-phase HPLC. It was noted that two of the isomers, in their 300-MHz ^1H NMR spectra, displayed well resolved signals for the C23, C24, and C28 protons, which appeared as an ABXY system. Inspection of the coupling constants and irradiation experiments revealed $J_{C23-C24}$ values of 5.3 Hz and 5.4 Hz, thus indicating that these two isomers **13a** and **13b** possessed a trans arrangement[13] between the hydroxymethyl group at C24 and the rest of the side chain at C23.

All of the marine cyclopropyl sterols isolated to date are known to possess the thermodynamically more stable trans arrangement of substituents around the cyclopropane ring.

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(2) Visiting investigator (1981–1983) from Shenyang College of Pharmacy and Liaoning Institute of Materia Medica, People's Republic of China.
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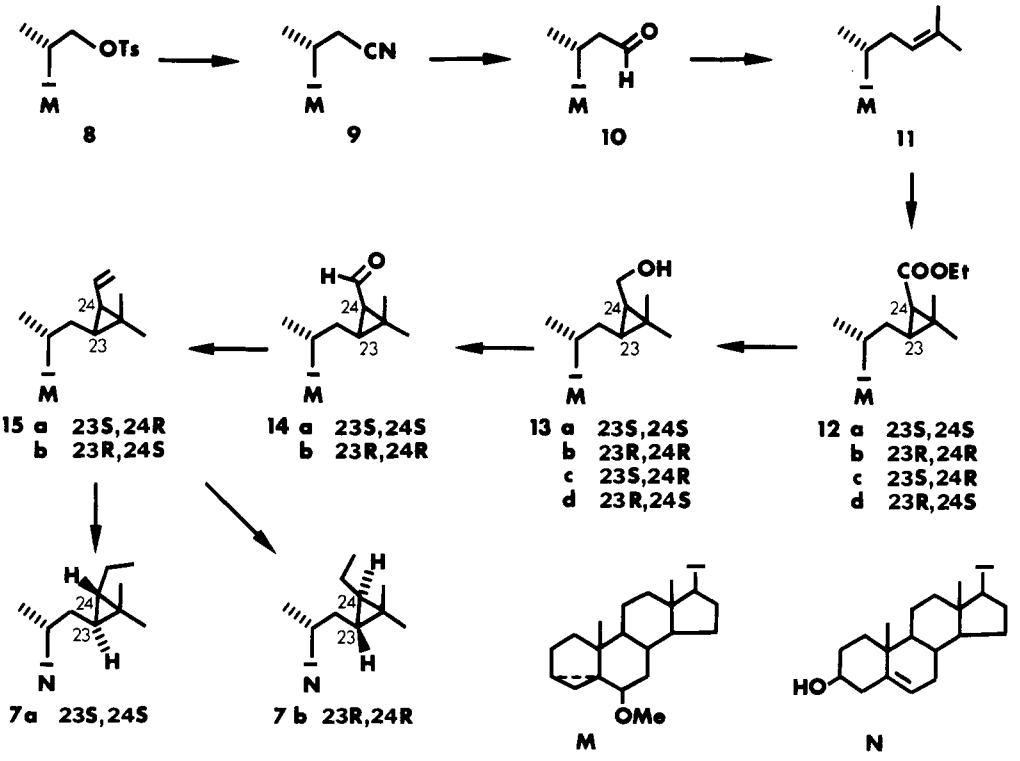
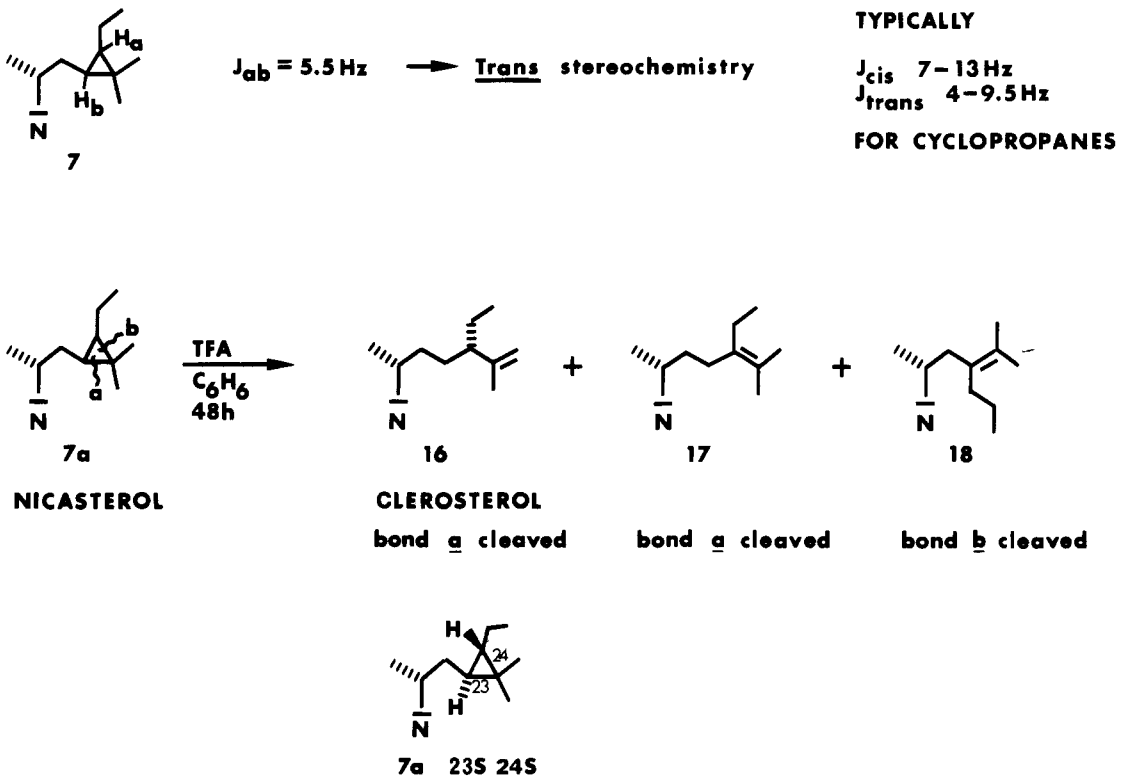
Scheme I. Synthesis of Nicasterol



Scheme II. Stereochemical Assignment of Nicasterol



It seemed likely that nicasterol would also possess this more stable substitution pattern and we therefore concentrated on the elaboration of the two trans isomers **13a** and **13b** to the proposed nicasterol structure.

Transformation of the hydroxymethyl groups of **13a** and **13b** to the ethyl group of nicasterol was accomplished via the aldehydes **14a** and **14b** and the olefins **15a** and **15b**. Reduction of the terminal methylene group of **15a** and **15b** with a Rhodium on charcoal catalyst proceeded quantitatively without any of the usual accompanying hydrogenolytic ring opening.[14] Cleavage of the *i*-methyl ether protecting group gave the free sterols **7a** and **7b**, one of which was identical with the natural product (Table I).

The absolute stereochemistry of the natural isomer was determined in a straightforward manner. Irradiation of the C28 methylene signal at δ 1.27 (located by decoupling the C29 methyl signal) collapsed the C24 cyclopropyl

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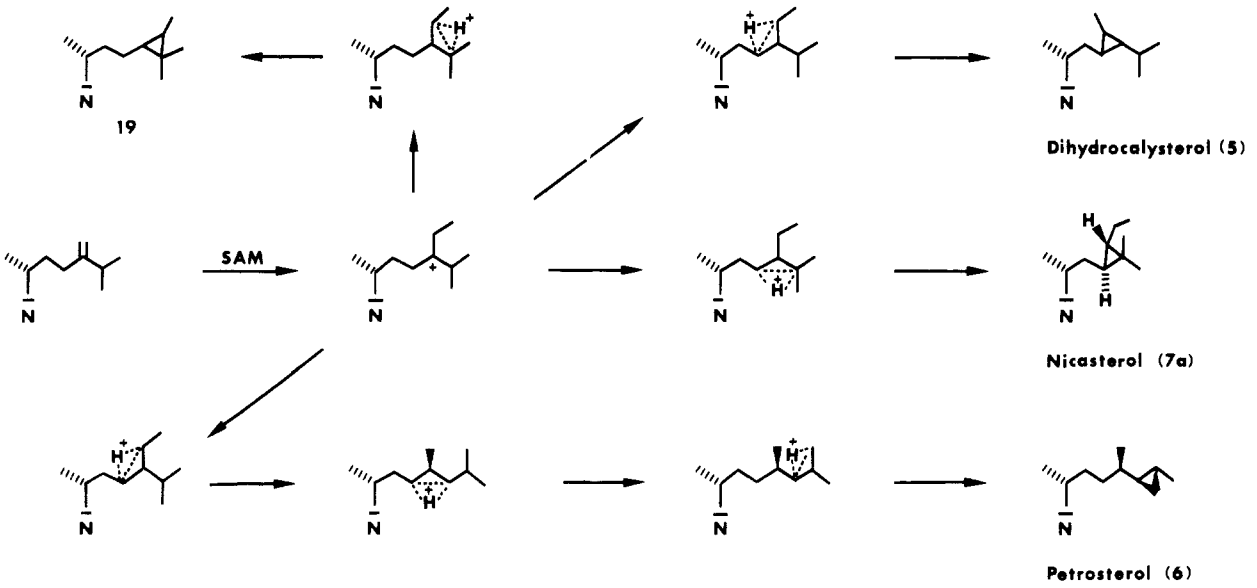
Table I. ^{1}H NMR Data of Nicasterol and Synthetic Compounds 7a and 7b

	nicasterol		7a		7b	
C	$CDCl_3$[a]	C_6D_6[a]	$CDCl_3$[b]	C_6D_6[b]	$CDCl_3$[b]	C_6D_6[b]
19	1.008	0.944	1.008	0.945	1.008	0.942
26[c]	1.001	1.082	1.001	1.079	0.998	1.096
27[c]	0.956	1.077	0.956	1.075	0.985	1.066
29	0.936 (7.0 Hz)	1.029 (7.3 Hz)	0.936 (7.3 Hz)	1.026 (7.3 Hz)	0.937 (7.3 Hz)	1.030 (7.3 Hz)
21	0.958 (6.3 Hz)	1.102 (6.6 Hz)	0.958 (6.3 Hz)	1.098 (6.4 Hz)	0.979 (6.5 Hz)	1.123 (6.4 Hz)
18	0.687	0.703	0.687	0.701	0.685	0.704
23	0.060	0.174	0.062 (5.5 Hz, 6.3 Hz, 6.4 Hz)	0.172	0.080	0.169
24	−0.022	0.084	−0.022 (5.5 Hz, 6.8 Hz, 6.2 Hz)	0.082	0.080	0.169

[a] 360-MHz ^{1}H NMR. [b] 300-MHz ^{1}H NMR. [c] Assignments are interchangeable.

Scheme III. Cyclopropylsterol Biosynthesis



proton signal at δ −0.024 to a doublet J = 5.5 Hz. This confirmed that the trans stereochemistry around the cyclopropane ring had been maintained throughout the synthetic sequence. Acid-catalyzed cyclopropane ring opening[15] (Scheme II) of the natural isomer gave, among other products, clerosterol (**16**).[16] Since the absolute stereochemistry at C24 is maintained during ring opening and since the cyclopropane ring is trans substituted, the absolute stereochemistry of nicasterol is that shown in **7a** (23S,24S).

Biosynthetically, nicasterol belongs to the family of cyclopropyl sterols arising from bioalkylation of 24-methylenecholesterol (Scheme III), which includes dihydrocalysterol (**5**) and petrosterol (**6**).[17] The mode of incorporation of 24-methylenecholesterol into petrosterol[17] is unclear at present but may involve the cyclopropane "walk" shown. Incorporation experiments with radiolabeled precursors are currently under way to test this hypothesis. One might also predict that **19**, arising from cyclopropane ring closure between C25 and C28 should also be found as a natural product.

(15) For other acid-catalyzed isomerizations of cyclopropyl sterols see: Lang, R. W.; Djerassi, C. *Tetrahedron Lett.* **1982**, *23*, 2063–2066. Lang, R. W.; Djerassi, C. *Helv. Chim. Acta* **1982**, *65*, 407–418. Catalan, C. A. N.; Djerassi, C. *Tetrahedron Lett.* **1983**, *24*, 3461–3464. Proudfoot, J. R.; Djerassi, C. *J. Am. Chem. Soc.* **1984**, *106*, 5613–5622. Zimmerman, M. P.; Li, H. T.; Duax, W. L.; Weeks, C. M.; Djerassi, C. *J. Am. Chem. Soc.* **1984**, *106*, 5602–5612.

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(17) Catalan, C. A. N., unpublished observation, this laboratory.

Experimental Details

High-performance liquid chromatography (HPLC) was carried out on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer). Two different reverse-phase systems were used: (i) A Whatman Partisil M9 10/50 ODS-2 column (9 mm i.d. × 10 cm) with absolute methanol as the mobile phase was used for the fractionation of the *Calyx nicaensis* sterol mixture. (ii) Two Altex Ultrasphere ODS 5-μm columns (10 mm i.d. × 25 cm) in series with absolute methanol as the mobile phase were used for the fractionation and purification of synthetic intermediates.

Analytical gas–liquid chromatography was performed on a Hewlett-Packard 402A gas chromatograph instrument with a flame-ionization detector and a U-shaped glass column (2 mm i.d. × 1.8 m) packed with 3% OV-17 on Gas Chrom Q. The oven temperature was 260 °C with helium as the carrier gas.

Fourier transform ^{1}H NMR spectra were recorded on a Bruker HXS-360 spectrometer operating at 360 MHz and equipped with a Nicolet TT 1010-A computer and also on a Nicolet Magnetics Corporation NMC-300 spectrometer equipped with a 1280 data system and operating at 300 MHz. All NMR spectra were referenced to either $CHCl_3$ (7.260 ppm) or C_6H_6 (7.150 ppm).

Low-resolution mass spectra were obtained with a Finnigan MAT-44 spectrometer in its GC–MS mode or with a Hewlett Packard 5995 spectrometer in either GC–MS or DI modes. High-resolution mass spectra were recorded on an MS-50 instrument at the University of California at Berkeley or on an MS-50 instrument at the Midwest Center for Mass Spectrometry, University of Nebraska at Lincoln. Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. Optical rotations were recorded in chloroform on a Perkin-Elmer 141 polarimeter.

The Isolation of Nicasterol (7a). The acetone extract of *Calyx nicaensis* was fractionated on an open silica gel column

(eluent hexane–ether 3/1). The sterol fractions (R_f cholesterol by TLC) were combined and subjected to reverse-phase HPLC to give, along with previously described sterols,[7] nicasterol (**7a**) as a trace component of the sterol mixture. GC relative retention time (rrt) 1.36 vs. cholesterol (3% OV 17, 260 °C). HPLC rrt 0.96 vs. cholesterol (Altex Ultrasphere ODS-5, methanol). ^{1}H NMR data are presented in Table I. High-resolution mass spectrum, m/z (relative intensity) 412.3727 (M^+ 9, calcd for $C_{29}H_{48}O$ 412.3705), 394.3603 ($C_{29}H_{46}$, 6), 314.2607 ($C_{22}H_{34}O$, 13), 300.2442 ($C_{21}H_{32}O$, 9), 299.2351 ($C_{21}H_{31}O$, 8), 283.2437 ($C_{21}H_{31}$, 12), 271.2071 ($C_{19}H_{27}O$, 29), 255.2097 ($C_{19}H_{27}$, 8), 245.1897 ($C_{17}H_{25}O$, 17), 231.1747 ($C_{16}H_{23}O$, 8), 229.1596 ($C_{16}H_{21}O$, 3), 215.1798 ($C_{16}H_{23}$, 8), 213.1652 ($C_{16}H_{21}$, 12), 57.0716 (C_4H_9, 100). There was insufficient material to record either a melting point or an optical rotation of the natural sterol. The relevant data were, therefore, secured with synthetic material.

20(*R*)-(Cyanomethyl)-6β-methoxy-3α,5-cyclo-5α-pregnane (9). A suspension of tosylate 8[12] (5.6 g, 11.2 mmol) and KCN (1.2 g, 18.5 mmol) in Me_2SO (30 mL) was warmed to 50 °C for 4 h. The reaction mixture was diluted with ethyl acetate, washed thoroughly with water, and dried ($MgSO_4$ anhydrous). Evaporation of the solvent gave the nitrile **9** (3.96 g, 99%) sufficiently pure for subsequent reactions: mp 84–85 °C; ^{1}H NMR (300 MHz) δ ($CDCl_3$) 3.324 (3 H, s, OCH_3), 2.353 (1 H, dd, J = 4 Hz, 16.7 Hz, C-22), 2.223 (1 H, dd, J = 6.9 Hz, 16.7 Hz, C-22), 1.160 (3 H, d, J = 6.7 Hz, C-21), 1.020 (3 H, s, C-19), 0.735 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 355 ($M^{+\cdot}$ 12, $C_{24}H_{37}ON$), 340 (32), 323 (44), 308 (14), 300 (66), 297 (10), 282 (5), 268 (5), 255 (5), 202 (10), 41 (100).

20(*R*)-(Formylmethyl)-6β-methoxy-3α,5-cyclo-5α-pregnane (10). A solution of the nitrile **9** (0.75 g, 2.14 mmol) in dry toluene was cooled to −78 °C and Dibal (1.2 M in hexane, 2 mL) was added all at once. The reaction mixture was allowed to warm to room temperature over a period of 2.5 h. Methanol (1 mL) was added followed by water (1 mL) and the mixture was stirred at room temperature for 20 min. Filtration and evaporation gave the crude aldehyde which was purified by chromatography over silica (eluent CH_2Cl_2) to give pure **10** (0.596 g 80%): ^{1}H NMR (300 MHz) δ ($CDCl_3$) 9.946 (1 H, m, C-23), 3.32 (3 H, s, OCH_3), 1.019 (3 H, s, C-19), 1.015 (3 H, d, J = 6.4 Hz, C-21), 0.765 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 358 ($M^{+\cdot}$ 57, $C_{24}H_{38}O_2$), 343 (69), 326 (100), 303 (98), 300 (36), 275 (32), 255 (22), 227 (30), 213 (41), 201 (35).

24-Methyl-26,27-dinor-6β-methoxy-3α,5-cyclo-5α-cholest-23-ene (11). To a suspension of isopropyltriphenylphosphonium iodide (432 mg, 1 mmol) in dry THF (10 mL) under N_2 at 0 °C was added dropwise *n*-butyllithium (2.6 M in hexane, 0.4 mL, 1 mmol). The resultant solution was stirred at 0 °C for 10 min. This phosphorane solution was then added dropwise, via a syringe, to a stirred solution of the aldehyde **10** (214 mg, 0.6 mmol) in dry THF at 0 °C under nitrogen until the red color persisted. After 1 h, methanol (0.5 mL) was added and the mixture was evaporated to dryness. Fractionation of the crude mixture over silica (eluent CH_2Cl_2–hexane, 1/4) gave the olefin **11** (154 mg, 62%): ^{1}H NMR (300 MHz) δ ($CDCl_3$) 5.120 (1 H, br t, C-23), 1.698 (3 H, s, C-26 or C-27), 1.586 (3 H, s, C-26 or C-27), 1.017 (3 H, s, C19), 0.888 (3 H, d, J = 6.6 Hz, C-21), 0.714 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 384 ($M^{+\cdot}$ 3, $C_{27}H_{44}O$), 369 (3), 352 (2), 329 (5), 300 (2), 285 (9), 283 (8), 277 (12), 253 (15), 227 (5), 215 (5), 213 (4), 201 (8), 55 (100).

Cyclopropanecarboxylic Acid Esters 12a–d. To the neat olefin **11** (50 mg, 0.13 mmol) and CuI (10 mg) in a 10-mL round-bottomed flask equipped with a magnetic stirrer and maintained at 55 °C on an oil bath was added ethyl diazoacetate (1 drop/15 min) until TLC indicated that no further starting material was being consumed. The reaction mixture was purified by elution from florisil (eluent CH_2Cl_2) and silica (eluent CH_2Cl_2–hexane gradient) to give a mixture of esters **12a–d** (33 mg) and recovered starting material (8 mg). The cyclopropyl esters were fractionated by HPLC (mobile phase, methanol) to give three fractions.

Fraction 1. (23*R*,24*R*)-24-(Ethoxycarbonyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (12b): yield 20% (by HPLC); ^{1}H NMR (300 MHz) δ ($CDCl_3$) 4.12 (2 H, m, $COOCH_2CH_3$), 3.320 (3 H, s, OCH_3), 1.255 (3 H, t, J = 7.2 Hz, $COOCH_2CH_3$), 1.211 (3 H, s, C-26 or C-27), 1.127 (3 H, s, C-26 or C-27), 1.018 (3 H, s, C-19), 0.978 (3 H, d, J = 6.42 Hz, C-21), 0.717 (3 H, s, C-18); high-resolution mass spectrum, m/z (relative intensity) 470.3743 ($M^{+\cdot}$ 6, calcd for $C_{31}H_{50}O_3$ 470.3760), 455.3526 ($C_{30}H_{47}O_3$, 11), 438.3519 ($C_{30}H_{46}O_2$, 25), 423.3267 ($C_{29}H_{43}O_2$, 10), 415.3199 ($C_{27}H_{43}O_3$, 23), 328.2776 ($C_{23}H_{36}O$, 4), 313.2537 ($C_{22}H_{33}O$, 6), 296.2484 ($C_{22}H_{32}$, 7), 285.2221 ($C_{20}H_{29}O$, 16), 283.2418 ($C_{21}H_{31}$, 15), 281.2279 ($C_{21}H_{29}$, 9), 255.2089 ($C_{19}H_{27}$, 32), 253.1952 ($C_{19}H_{25}$, 100), 243.2117 ($C_{18}H_{27}$, 11), 227.1792 ($C_{17}H_{23}$, 16), 213.1648 ($C_{16}H_{21}$, 20), 201.1642 ($C_{15}H_{21}$, 10).

Fraction 2 was a mixture of **(23*S*,24*S*)-24-(ethoxycarbonyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (12a)** and one of the *cis*-cyclopropane isomers **12c** or **12d**: yield 33% (by HPLC); mass spectrum, m/z (relative intensity) 470 ($M^{+\cdot}$ 4), 455 (11), 438 (7), 423 (2), 415 (9), 328 (7), 313 (5), 296 (7), 285 (23), 283 (8), 281 (6), 255 (21), 253 (100), 243 (3), 227 (12), 213 (14), 201 (8).

Fraction 3. (23ξ,24ξ)-24-(Ethoxycarbonyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (12c or 12d): yield 12% (by HPLC); ^{1}H NMR (300 MHz) δ ($CDCl_3$) 4.08 (2 H, m, $COOCH_2CH_3$), 3.313 (3 H, s, OCH_3), 1.242 (3 H, t, J = 7.1 Hz, $COOCH_2CH_3$), 1.189 (3 H, s, C-26 or C-27), 1.142 (3 H, s, C-26 or C-27), 1.013 (3 H, s, C-19), 0.928 (3 H, d, J = 6.4 Hz, C-21), 0.703 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 470 ($M^{+\cdot}$ 6), 455 (6), 438 (8), 423 (2), 415 (11), 328 (9), 313 (7), 296 (10), 285 (25), 283 (8), 281 (6), 255 (27), 253 (100), 243 (3), 227 (14), 213 (17), 201 (9).

General Procedure for the Reduction of Esters 12a–d to the Corresponding Alcohols 13a–d. The cyclopropyl ester (24 mg, 0.05 mmol) was dissolved in dry ether (2 mL) and lithium aluminium hydride (6 mg) was added. After 12 h the reaction was quenched by the addition of ethyl acetate and water and filtered. Evaporation followed by purification over silica (eluent CH_2Cl_2) gave the corresponding alcohol (19 mg, 90%). From **12b** was obtained **(23*R*,24*R*)-24-(hydroxymethyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (13b)**: ^{1}H NMR (300 MHz) δ ($CDCl_3$) 3.789 (1 H, dd, J = 6.1, 11.4 Hz, C-28), 3.475 (1 H, dd, J = 8.9, 11.4 Hz, C-28), 3.320 (3 H, s, OCH_3), 1.099 (3 H, s, C-26 or C-27), 1.054 (3 H, s, C-26 or C-27), 1.018 (3 H, s, C-19), 0.972 (3 H, d, J = 6.5 Hz, C-21), 0.718 (3 H, s, C-18), 0.572 (1 H, ddd, J = 5.4, 6.1, 8.9 Hz, C-24), 0.372 (1 H, br q, C-23); high-resolution mass spectrum, m/z (relative intensity) 428.3660 ($M^{+\cdot}$ 2, calcd for $C_{29}H_{48}O_2$ 428.3654), 413.3408 ($C_{28}H_{45}O_2$, 7), 396.3381 ($C_{28}H_{44}O$, 11), 378.3291 ($C_{28}H_{42}$, 12), 373.3097 ($C_{25}H_{41}O$, 12), 365.3184 ($C_{27}H_{41}$, 5), 363.3040 ($C_{27}H_{39}O$, 5), 355.3006 ($C_{25}H_{39}O$, 12), 328.2758 ($C_{23}H_{36}O$, 5), 313.2536 ($C_{22}H_{33}O$, 5), 296.2486 ($C_{22}H_{32}$, 9), 285.2216 ($C_{20}H_{29}O$, 15), 283.2411 ($C_{21}H_{31}$, 13), 255.2093 ($C_{19}H_{27}$, 16), 253.1948 ($C_{19}H_{25}$, 76), 227.1793 ($C_{17}H_{23}$, 15), 213.1646 ($C_{16}H_{21}$, 19), 81.0687 (C_6H_9, 100).

From the mixture of **12a** and **12c** or **12d** was obtained **13a** and **13c** or **13d**.

(23*S*,24*S*)-24-(Hydroxymethyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (13a): ^{1}H NMR (300 MHz) δ ($CDCl_3$) 3.754 (1 H, dd, J = 6.4, 11.4 Hz, C-28), 3.490 (1 H, dd, J = 8.2, 11.4 Hz, C-28), 3.322 (3 H, s, OCH_3), 1.104 (3 H, s, C-26 or C-27), 1.027 (3 H, s, C-26 or C-27), 1.021 (3 H, s, C-19), 0.949 (3 H, d, J = 6.6 Hz, C-21), 0.723 (3 H, s, C-18), 0.502 (1 H, ddd, J = 8.2, 6.4, 5.4 Hz, C-24), 0.325 (1 H, br q, C-23); mass spectrum, m/z (relative intensity) 428 ($M^{+\cdot}$ 3), 413 (6), 396 (8), 373 (14), 365 (5), 363 (1), 355 (2), 328 (4), 313 (8), 296 (5), 285 (26), 283 (16), 255 (16), 253 (100), 227 (15), 213 (15).

***cis*-(23ξ,24ξ)-24-(Hydroxymethyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (13c or 13d)**: ^{1}H NMR (300 MHz) δ ($CDCl_3$) 3.627 (2 H, m, C-28), 3.320 (3 H, s, OCH_3), 1.080 (3 H, s, C-26 or C-27), 1.020 (3 H, s, C-26 or C-27), 1.007 (3 H, s, C-19), 0.967 (3 H, d, J = 6.5 Hz, C-21), 0.726 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 428 ($M^{+\cdot}$ 4), 413 (4), 396 (12), 373 (15), 365 (8), 363 (12), 355 (8), 328 (8), 313 (8), 285 (30), 283 (15), 255 (11), 253 (100), 227 (19), 213 (19).

From **12c** or **12d** was obtained **13c** or **13d**.

***cis*-(23ξ,24ξ)-24-(Hydroxymethyl)-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (13c or 13d)**: ^{1}H NMR (300 MHz) δ ($CDCl_3$) 3.672 (1 H, dd, J = 7.1, 11.3 Hz, C-28), 3.550 (1 H, dd, J = 8.2, 11.3 Hz, C-28), 3.321 (3 H, s, OCH_3), 1.083 (3 H, s, C-26 or C-27), 1.020 (3 H, s, C-19), 0.988 (3 H, s, C-26 or C-27), 0.946 (3 H, d, J = 6.5 Hz, C-21), 0.721 (3 H, s, C-18), 0.553 (1 H, ddd, J = 6.0, 7.3, 9.0 Hz, C-23); mass spectrum, m/z (relative intensity)

428 ($M^{+\cdot}$ 5), 413 (8), 396 (10), 373 (18), 365 (6), 363 (1), 355 (3), 328 (6), 313 (4), 285 (30), 283 (20), 255 (21), 253 (100), 227 (18), 213 (19).

General Procedure for the Conversion of Alcohols 13a and 13b to Olefins 15a and 15b. To a stirred solution of the alcohol **13a** or **13b** (12.5 mg, 0.03 mmol) in CH_2Cl_2 (1 mL) was added pyridinium chlorochromate (20 mg, 0.1 mmol). After 1 h, the reaction mixture was filtered through Florisil (eluent CH_2Cl_2) and evaporated to give the pure aldehyde **14a** or **14b**. After drying under vacuum overnight, the aldehyde was dissolved in dry THF (1 mL) at 0 °C and a preformed solution of methylenetriphenylphosphorane was added dropwise until the orange color persisted. After 1 h, methanol (0.1 mL) was added, and the mixture was evaporated to dryness. Fractionation over silica (eluent CH_2Cl_2–hexane gradient) followed by reverse-phase HPLC (mobile phase, methanol) gave the pure olefin **15a** or **15b**.

(23*R*,24*R*)-24-Formyl-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (14b): ^1H NMR (300 MHz) δ ($CDCl_3$) 9.316 (1 H, d, J = 5.8 Hz, C-28), 3.321 (3 H, s, OCH_3), 1.294 (3 H, s, C-26 or C-27), 1.163 (3 H, s, C-26 or C-27), 1.018 (3 H, s, C-19), 0.983 (3 H, d, J = 6.4 Hz, C-21), 0.710 (3 H, s, C-18).

(23*S*,24*S*)-24-Formyl-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (14a): ^1H NMR (300 MHz) δ ($CDCl_3$) 9.303 (1 H, d, J = 4.6 Hz, C-28), 3.318 (3 H, s, OCH_3), 1.298 (3 H, s, C-26 or C-27), 1.136 (3 H, s, C-26 or C-27), 1.015 (3 H, s, C-19), 0.946 (3 H, d, J = 6.5 Hz, C-21), 0.715 (3 H, s, C-18).

(23*R*,24*S*)-24-Vinyl-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (15b): ^1H NMR (300 MHz) δ ($CDCl_3$) 5.553 (1 H, ddd, J = 9.2, 10.1, 17.0 Hz, C-28), 5.025 (1 H, dd, J = 2.0, 17.0 Hz, C-29), 4.914 (1 H, dd, J = 2.0, 10.1 Hz, C-29), 3.322 (3 H, s, OCH_3), 1.046 (6 H, s, C-26, C-27), 1.019 (3 H, s, C-19), 0.968 (3 H, d, J = 6.5 Hz, C-21), 0.721 (3 H, s, C-18), high-resolution mass spectrum, m/z (relative intensity) 424.3697 ($M^{+\cdot}$ 0.25, $C_{30}H_{48}O$ requires 424.3705), 409.3482 ($C_{29}H_{45}O$, 1), 392.3458 ($C_{29}H_{44}$, 7), 377.3214 ($C_{28}H_{41}$, 9), 369.3146 ($C_{26}H_{41}O$, 2), 283.2432 ($C_{21}H_{31}$, 8), 253.1949 ($C_{19}H_{25}$, 53), 227.1794 ($C_{17}H_{23}$, 5), 213.1644 ($C_{16}H_{21}$, 10), 95.0858 (C_7H_{11}, 100).

(23*S*,24*R*)-24-Vinyl-6β-methoxy-3α,5:23,25-dicyclo-5α-cholestane (15a): ^1H NMR (300 MHz) δ ($CDCl_3$) 5.555 (1 H, ddd, J = 9.1, 9.9, 16.8 Hz, C-28), 5.022 (1 H, dd, J = 1.9, 16.9 Hz), 4.908 (1 H, dd, J = 2.0, 10.1 Hz), 3.322 (3 H, s, OCH_3), 1.047 (3 H, s, C-26 or C-27), 1.017 (6 H, s, C-19, C-26 or C-27), 0.945 (3 H, d, J = 6.6 Hz, C-21), 0.721 (3 H, s, C-18); high-resolution mass spectrum, m/z (relative intensity) 424.3666 ($M^{+\cdot}$ 3, $C_{30}H_{48}O$ requires 424.3705), 409.3518 ($C_{29}H_{45}O$, 4), 392.3356 ($C_{29}H_{44}$, 6), 369.3149 ($C_{26}H_{41}O$, 5), 283.2432 ($C_{21}H_{31}$, 14), 253.1958 ($C_{19}H_{25}$, 100), 227.1839 ($C_{17}H_{23}$, 6), 213.1646 ($C_{16}H_{21}$, 5).

General Procedure for the Reduction of Olefins 15a and 15b and Cleavage of the *i*-Methyl Ether Protecting Group. The olefin **15a** or **15b** (6 mg) was dissolved in methanol (2 mL) and 5% Rh/C (5 mg) was added. The mixture was stirred under hydrogen for 2 h, filtered, and evaporated to dryness. The residue was taken up in dioxan–water (1/1, 1 mL) and a crystal of *p*-toluenesulfonic acid was added. The reaction mixture was heated under reflux until no starting material remained (TLC monitoring). Evaporation of the solvents and purification over silica (eluent CH_2Cl_2) gave the free sterols **7a** or **7b** (4 mg, 66%).

(23*S*,24*S*)-23,25-Cyclostigmast-5-en-3β-ol (nicasterol) (7a): mp 132–134 °C (MeOH); $[\alpha]^{20}_D$ –25.8 (c 1.55); ^1H NMR (300 MHz) data are reported in Table I; high-resolution mass spectrum, m/z (relative intensity) 412.3744 ($M^{+\cdot}$ 18, calcd for $C_{29}H_{48}O$ 412.3705), 394.3551 ($C_{29}H_{46}$, 9), 314.2741 ($C_{22}H_{34}O$, 12), 300.2454 ($C_{21}H_{32}O$, 36), 299.2362 ($C_{21}H_{31}O$, 18), 283.2412 ($C_{21}H_{31}$, 13), 281.2336 ($C_{21}H_{29}$, 13), 271.2122 ($C_{19}H_{27}O$, 47), 255.2163 ($C_{19}H_{27}$, 12.5), 231.1823 ($C_{16}H_{23}O$, 7), 229.1643 ($C_{16}H_{21}O$, 5), 215.1805 ($C_{16}H_{23}$, 18), 213.1615 ($C_{16}H_{21}$, 9), 97.1022 (C_7H_{13}, 100).

(23*R*,24*R*)-23,25-Cyclostigmast-5-en-3β-ol (7b): mp 128–130 °C (MeOH); $[\alpha]^{20}_D$ –30.0 (c 0.6); ^1H NMR (300 MHz) data are reported in Table I; mass spectrum, m/z (relative intensity) 412 ($M^{+\cdot}$ 2), 394 (2), 314 (18), 300 (10), 299 (6), 283 (7), 281 (6), 271 (42), 255 (7), 253 (15), 231 (5), 229 (9), 215 (11), 213 (16), 97 (100).

Acid-Catalyzed Isomerization of Nicasterol. Nicasterol (**7a**) (1.5 mg) was dissolved in benzene (1 mL) and trifluoroacetic acid (0.05 mL) was added. The reaction mixture was left undisturbed at room temperature for 70 h. The solvents were evaporated and the residue in ether (2 mL) was treated with lithium aluminum hydride (5 mg) at room temperature for 1 h. Excess reducing agent was destroyed with ethyl acetate and water and the reaction mixture was filtered, evaporated, and passed through a short silica plug (eluent CH_2Cl_2). Fractionation by HPLC (reverse phase, eluent MeOH) gave three compounds, each in about 20% yield.

Fraction 1. Clerosterol (16): ^1H NMR (300 MHz) δ ($CDCl_3$) 5.356 (1 H, m, C-6), 4.723 (1 H, br s, C-26), 4.642 (1 H, br s, C-26), 1.007 (3 H, s, C-19), 0.903 (3 H, d, J = 6.4 Hz, C-21), 0.798 (3 H, t, J = 7.4 Hz, C-29), 0.669 (3 H, s, C-18); ^1H NMR (300 MHz) δ (C_6D_6) 5.346 (1 H, m, C-6), 4.873 (1 H, br s, C-26), 4.825 (1 H, br s, C-26), 1.573 (3 H, s, C-27), 1.000 (3 H, d, J = 6.5 Hz, C-21), 0.944 (3 H, s, C-19), 0.886 (3 H, t, J = 7.5 Hz, C-29), 0.676 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 412 (M^+ 27, $C_{29}H_{48}O$), 397 (3), 379 (3), 328 (3), 314 (17), 300 (10), 299 (20), 281 (7), 272 (13), 271 (26), 258 (10), 255 (10), 253 (10), 231 (10), 229 (17), 213 (23), 211 (17).

Fraction 2. Stigmasta-5,24(25)-dien-3β-ol (17): ^1H NMR (300 MHz) δ ($CDCl_3$) 5.36 (1 H, m, C-6), 1.008 (3 H, s, C-19), 0.966 (3 H, d, J = 6.7 Hz, C-21), 0.930 (3 H, t, J = 7.5 Hz, C-29), 0.681 (3 H, s, C-18); ^1H NMR (300 MHz) δ (C_6D_6) 5.35 (1 H, m, C-6), 1.712 (3 H, s, C-26 or C-27), 1.658 (3 H, s, C-26 or C-27), 1.057 (3 H, d, J = 6.2 Hz, C-21), 1.047 (3 H, t, J = 7.4 Hz, C-29), 0.943 (3 H, s, C-19), 0.660 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 412 (M^+ 7, $C_{29}H_{48}O$), 314 (53), 300 (7), 299 (18), 296 (7), 281 (20), 271 (28), 255 (7), 253 (9), 239 (7), 231 (11), 229 (33), 213 (21), 211 (21).

Fraction 3. 23-Propyl-26,27-dinor-24-methylcholesta-5,23(24)-dien-3β-ol (18): ^1H NMR (300 MHz) δ ($CDCl_3$) 5.36 (1 H, m, C-6), 1.010 (3 H, s, C-19), 0.879 (3 H, t, J = 7.2 Hz, C-31), 0.814 (3 H, d, J = 6.5 Hz, C-21), 0.695 (3 H, s, C-18); ^1H NMR (300 MHz) δ (C_6D_6) 5.35 (1 H, m, C-6), 1.708 (6 H, s, C-28 and C-25), 1.003 (3 H, d, J = 6.5 Hz, C-21), 0.937 (3 H, s, C-19), 0.939 (3 H, t, J = 7.4 Hz, C-31), 0.701 (3 H, s, C-18); mass spectrum, m/z (relative intensity) 412 (M^+ 4, $C_{29}H_{48}O$), 314 (4), 301 (20), 300 (60), 285 (15), 283 (29), 282 (10), 271 (17), 267 (11), 255 (4), 253 (4), 241 (13), 227 (10), 215 (32), 213 (18), 201 (11).

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