Synthesis of the Four Stereoisomers of 22,23-Dimethylcholesterol

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22,23-Dimethylcholesterol, though hitherto unknown, is likely to exist in nature. In order to facilitate its recognition all four stereoisomers have been synthesized.

A unique feature of certain marine sterols—never encountered among terrestrial counterparts—is the occurrence of bioalkylation of the cholesterol side chain at positions 22 and 23. Gorgosterol (**5**)[1] is the first recorded example and we hypothesized[1-3] that its biosynthetic precursor is brassicasterol (**3**), itself derived by the conventional C-24 alkylation from a Δ^{24}-sterol such as desmosterol (**1**).[4,5] A different site of alkylation, namely, direct attack of the Δ^{22} double bond of cholesterol, is also possible.[2,3] The isolation of (22*R*,23*R*)-22,23-methylenecholesterol (**10**),[6] 22-methylenecholesterol (**11**),[7] and 23-methyl-22,23-dehydrocholesterol (**8**)[8,9] and its 4α-methyl analogue[10] provided indirect evidence for such direct alkylation of a Δ^{22} double bond of a sterol side chain. Presumably the first biosynthetic step is methylation by S-adenosylmethionine (SAM) of 22,23-dehydrocholesterol (**6**) to give **7** or **9** which on loss of a C-28 proton produces the cyclopropane **10**. Loss of a C-23 proton from **7** would lead to **8** whereas loss of a C-22 proton from **9** could lead to the hitherto unknown olefin **12**. The formation of 22-methylenecholesterol (**11**) is likely to occur through a 1,2-hydrogen shift to the more stable cation **13**, followed by loss of a C-28 proton.

The isolation of 22-methylenecholesterol (**11**)[7] and 23-methyl-22,23-dehydrocholesterol (**8**)[8,9] suggests that further bioalkylation products such as 22,23-dimethylcholesterol (**14**) will eventually be found in the marine environment. This could arise by various paths such as SAM biomethylation of the naturally occurring sterol **8** or of the hitherto unknown sterol **12**. To expedite their eventual isolation, we have undertaken the synthesis of all four stereoisomers in order to provide authentic reference compounds and to indicate which physical method would be of greatest diagnostic utility.

The synthesis (Scheme II) started with the aldehyde **15**, which was prepared from stigmasterol by following procedures described in the literature.[11,12] Addition of methylmagnesium iodide to the aldehyde **15** furnished the epimeric alcohols **16** which were oxidized with Jones reagent to give the ketone **17**. Wittig reaction of the ketone **17** with isopentyltriphenylphosphonium bromide (**18**) afforded the olefins **19**, which on hydroboration yielded the epimeric alcohols **20**. Oxidation of **20** with Jones reagent furnished the ketone **21**, olefination of which was achieved with methyltriphenylphosphonium bromide; the resulting

Scheme I

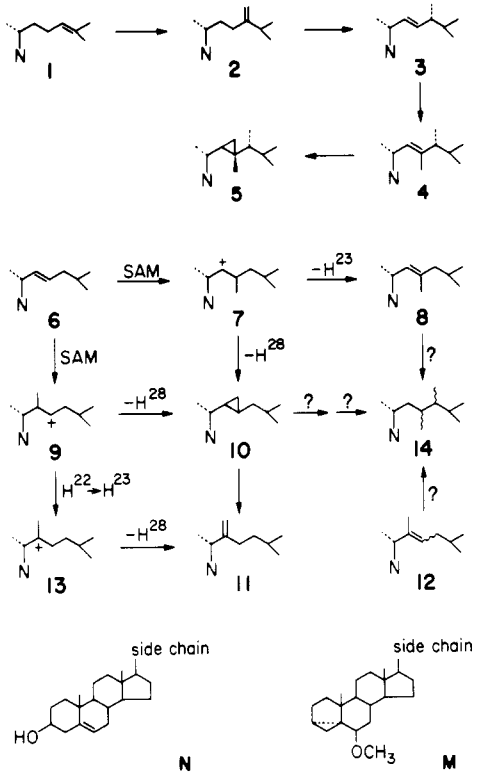
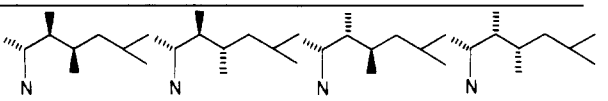


Table I. 300-MHz Proton NMR Data for the Methyl Groups of the Isomeric 22,23-Dimethylcholesterols 24, 25, 28, and 29[a]



Me	**24** (22*S*,23*R*)[b]	**25** (22*S*,23*S*)[b]	**28** (22*R*,23*R*)[b]	**29** (22*R*,23*S*)[b]
C-18	0.683	0.679	0.634	0.653
C-19	1.004	1.002	1.010	1.007
C-21	0.738 (6.68)	0.749 (6.61)	0.744 (6.74)	0.732 (7.26)
C-26	0.892 (6.50)[c]	0.891 (6.58)[c]	0.869 (6.87)[c]	0.850 (6.81)[c]
C-27	0.817 (6.41)[c]	0.817 (6.31)[c]	0.809 (6.62)[c]	0.814 (6.69)[c]
C-28	0.817 (6.41)	0.836 (5.37)	0.759 (6.73)	0.758 (7.01)
C-29	0.665 (6.76)	0.659 (6.80)	0.759 (6.73)	0.797 (6.68)

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b] The configuration at C-23 is reversible—see ref 16. [c] The assignment could be reversed.

(1) Ling, N. C.; Hale, R. L.; Djerassi, C. *J. Am. Chem. Soc.* **1970**, *92*, 5281-5282.
(2) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815-1828.
(3) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873-890.
(4) (a) Lederer, E. *Q. Rev. Chem. Soc.* **1969**, *23*, 453-481. (b) Nes, W. R.; McKean, M. L. "Biochemistry of Steroids and Other Isopentenoids"; University Park Press: Baltimore, MD, 1977.
(5) Goad, L. J. In "Marine Natural Products"; Vol II, Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. II, pp 75-172.
(6) Blanc, P. A.; Djerassi, C. *J. Am. Chem. Soc.* **1980**, *102*, 7113-7114; **1981**, *103*, 7036.
(7) Zielinski, J.; Li, H. T.; Milkova, T. S.; Popov, S.; Marekov, N. L.; Djerassi, C. *Tetrahedron Lett.* **1981**, *22*, 2345-2348.
(8) Kokke, W. C. M. C.; Withers, N. W.; Massey, I. J.; Fenical, W. H.; Djerassi, C. *Tetrahedron Lett.* **1979**, 3601-3604.
(9) Kobayashi, M.; Tomioka, A.; Hayashi, T.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1979**, *27*, 1951-1953.
(10) Alam, M.; Schram, K. H.; Ray, S. M. *Tetrahedron Lett.* **1978**, 3517-3518.
(11) Hutchins, R. F. N.; Thompson, J. J.; Svoboda, J. A. *Steroids* **1970**, *15*, 113-130.
(12) (a) Salmond, W. J.; Sobala, M. C. *Tetrahedron Lett.* **1977**, 1695-1698. (b) Anderson, G. D.; Powers, T. J.; Djerassi, C.; Fayos, J.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 388-394.

0022-3263/85/1950-0154$01.50/0

Scheme II

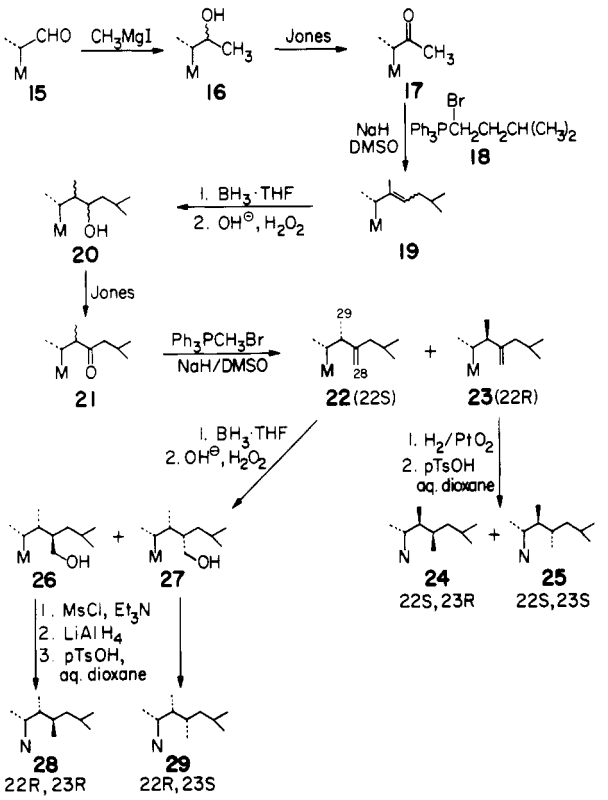


Table II. Physical Constants of 22,23-Dimethylcholesterols

sterol[16]	mp, °C	GC relative t_R[a] (3% SP-2250)	HPLC relative t_R[a] (Altex-ultrasphere)
(22*S*,23*R*)-**24**	121–122	1.25	1.00
(22*S*,23*S*)-**25**	159–160	1.38	1.08
(22*R*,23*R*)-**28**		1.20	1.26
(22*R*,23*S*)-**29**	142–143	1.25	1.26

[a] Cholesterol t_R = 1.00.

olefins **22** and **23** were separated by HPLC by using silver nitrate in the mobile phase.[13] The C-22 *R* stereochemistry of **23** was deduced from the characteristic[14,15] upfield shift of the C-29 methyl in the proton NMR spectrum.

Hydrogenation of **23** with Adam's catalyst followed by the removal of the *i*-methyl ether protecting group gave the two HPLC-separable 22,23-dimethylcholesterols **24** and **25**.[16] The hydrogenation products from **22** were not separable by HPLC; hydroboration of **22** gave the epimeric alcohols **26** and **27** which were separable by thin layer chromatography. Lithium aluminum hydride reduction of the mesylate of each pure alcohol followed by the removal of the *i*-methyl ether protecting group afforded the pure 22,23-dimethylcholesterols **28** and **29**.[16]

Table I summarizes the proton NMR spectra of the methyl region of the four stereoisomers of 22,23-dimethylcholesterol. The assignment of the C-28 methyl signals was based on the selective introduction of deuterium[15] by using lithium aluminum deuteride to reduce the mesylates of the alcohols derived from the olefins **22** and **23**. Although the physical constants of the four stereoisomers (Table II) show that differences in melting points and chromatographic mobilities exist among them, the NMR spectra provide the most reliable means of differentiation. As expected the mass spectra of all four isomers are identical. The availability of these synthetic reference compounds and their physical and spectral properties will greatly facilitate the search for such compounds from natural marine sources. The stereochemical uncertainty[16] at C-23 does not present a problem since all four possible 22,23-dimethylcholesterol isomers are available. However, it should be noted that we assume that no isomerization has occurred at C-20 during the synthesis, notably in the Wittig condensation of **17**. This has not been a problem in earlier reported[17] Wittig condensations of C-22 carbonyl compounds although there has been one report indicating some isomerization at C-20 during a Wittig reaction of a C-22 ketone.[7] However, the NMR data (Table I) tend to support the assumption that no isomerization at C-20 occurred during the Wittig condensation of **17** since the C-21 methyl signals are all within δ 0.049 (ppm) whereas steroids epimeric at C-20 show much larger differences $\delta_{20_\beta} - \delta_{20_\alpha}$ = 0.08–0.16 ppm.[17]

Experimental Section

General Methods. Melting points were determined with a Kofler hot stage apparatus. Gas chromatography was performed on a U-shaped glass column packed with 3% SP-2250 at 260 °C. This column was mounted on a Hewlett-Packard 402 high efficiency gas chromatograph equipped with a flame-ionization detector. HPLC was performed on a Waters Associates HPLC system (M 6000 pump, R 403 Differential refractometer and a Whatman Partisil M9 10/50 ODS-2 or an Altex-ultrasphere column) with methanol as the mobile phase.

Proton NMR spectra were recorded on a Nicolet NMC 300-MHz wide bore spectrometer or a Joel GX 500 MHz instrument with $CDCl_3$ or C_6D_6 as solvent. Chemical shifts are given in parts per million and coupling constants in hertz. High-resolution mass spectral data were obtained on an MS 50 instrument (University of California, Berkeley) and combined GC/MS was performed on a Hewlett-Packard HP 5995 instrument.

6β-Methoxy-3α,5-cyclo-5α-24-norcholan-22-one (17). The aldehyde **15** (prepared according to literature procedures[11,12] from stigmasterol *i*-methyl ether, 1.5 g, 4.4 mmol) in 15 mL of ether was added to the Grignard, formed from 486 mg (20 mmol) of magnesium and 2.84 g (20 mmol) of methyl iodide in ether, and the mixture stirred overnight at room temperature. After the usual workup, the crude alcohol **16** obtained was taken up in 50 mL of acetone and oxidized with Jones reagent at 0–5 °C. The usual workup followed by purification through preparative TLC (silica gel, 8:2 hexane/ethyl acetate as eluent) afforded the ketone **17** in 46% yield from aldehyde **15**: NMR δ 0.727 (3 H, s, 18-CH_3), 1.010 (3 H, s, 19-CH_3), 1.107 (3H, d, J = 6.92 Hz, 21-CH_3), 2.093 (3 H, s, 23-CH_3), 3.306 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 358 (53, M), 343 (24), 326 (100), 311 (10), 303 (58), 300 (21), 239 (11), 213 (10).

6β-Methoxy-22-methyl-3α,5-cyclo-5α-cholest-22-ene (19). Dry Me_2SO (10 mL) was added to oil-free sodium hydride (144 mg, 6 mmol) at room temperature under nitrogen atmosphere. The flask was heated (45–50 °C) until all evolution of hydrogen ceased (1 h). After cooling to room temperature a solution of (3-methylbutyl)triphenylphosphonium bromide (**18**) (2.9 g, 7 mmol) in 10 mL of dry Me_2SO was added and the solution stirred for 1 h. A solution of the ketone **17** (0.5 g, 1.4 mmol) in 2 mL of dry THF was introduced and the mixture heated for 48 h at 75–80 °C under nitrogen atmosphere. After the usual workup and purification by preparative TLC (silica gel, 9:1 hexane/ethyl acetate) the olefin **19** was obtained in 50% yield: NMR δ (C_6D_6) 0.736, 0.771 (3 H, s, 18-CH_3), 0.924, 0.926 (3 H, d, J = 6.53, 5.53 Hz, 21-CH_3), 0.943, 0.938 (3 H, d, J = 6.34, 6.45 Hz, 27-CH_3), 0.971, 0.980 (3 H, d, J = 6.84, 6.76 Hz, 26-CH_3), 1.737 (3 H, s, 28-CH_3), 3.215 (3 H, s, 6-OCH_3), 5.08–5.15, 5.26–5.32 (1 H, t, 23-CH); mass spectrum, m/z (relative intensity) 412 (44, M), 397 (24), 380 (26),

(13) Phelan, P. L.; Miller, J. R. *J. Chromatogr. Sci.* **1981**, *19*, 13–17.
(14) Zielinski, J.; Li, H. T.; Djerassi, C. *J. Org. Chem.* **1982**, *47*, 620–625.
(15) Gebreyesus, T.; Djerassi, C. *J. Org. Chem.* **1984**, *49*, 987–991.
(16) The actual stereochemistry at C-23 has not been established and the present assignment is arbitrary.
(17) Piatak, D. M.; Wicha, J. *Chem. Rev.* **1978**, *78*, 199–241.

357 (49), 255 (100), 229 (28), 213 (24).

6β-Methoxy-22-methyl-3α,5-cyclo-5α-cholestan-23-one (21). A solution of 200 mg (0.49 mmol) of **19** in 10 mL of dry THF was cooled in an ice bath under nitrogen and 15 mL of an approximately 1 M solution of diborane in THF was added with stirring. The stirring was continued for 1 h in the ice bath and then at room temperature overnight. The mixture was cooled again and 10 mL of water was added dropwise, followed by 10 mL of 3 N sodium hydroxide, and finally by the slow addition of 10 mL of 30% hydrogen peroxide. The mixture was stirred at room temperature for 3 h and then extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed successively with water and saturated sodium chloride solution and dried over magnesium sulfate. After evaporation of the solvent, the crude alcohols **20** were taken up in acetone (30 mL) and oxidized with Jones reagent at 0–5 °C. The usual workup followed by preparative TLC (silica gel, 8:2 hexane/ethyl acetate as eluent) afforded 115 mg (56% from the olefin **19**) of the ketone **21**. The epimeric ketone **21** could be separated by HPLC (Altex-ultrasphere, 98:2 methanol/water as eluent).

Less polar ketone: NMR δ 0.719 (3 H, s, 18-CH_3), 0.747 (3 H, d, J = 6.88 Hz, 28-CH_3), 0.826 (3 H, d, J = 6.62 Hz, 21-CH_3), 1.013 (3 H, s, 19-CH_3), 1.075 (3 H, d, J = 6.97 Hz, 27-CH_3), 1.077 (3 H, d, 6.84 Hz, 26-CH_3), 3.320 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 413 (3), 396 (3), 373 (6), 314 (5), 283 (100), 282 (58), 259 (14), 253 (16), 213 (14).

More polar ketone: NMR δ 0.670 (3 H, s, 18-CH_3), 0.901 (3 H, d, J = 6.51 Hz, 21-CH_3), 0.913 (6 H, d, J = 6.76 Hz, 26- and 27-CH_3), 1.004 (3 H, d, J = 7.85 Hz, 28-CH_3), 1.017 (3 H, s, 19-CH_3), 3.320 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 428 (40, M), 413 (26), 396 (35), 373 (62), 342 (57), 284 (52), 255 (37), 229 (30), 213 (100).

6β-Methoxy-22-methyl-23-methylene-3α,5-cyclo-5α-cholestane (22 and 23). Dry Me_2SO (5 mL) was added to oil-free sodium hydride (25 mg, 1 mmol) at room temperature under nitrogen atmosphere. The flask was heated (45–50 °C) until all evolution of hydrogen ceased (35 min). After cooling to room temperature a solution of methyltriphenylphosphonium bromide (428 mg, 1.2 mmol) in 2 mL of Me_2SO was added and the solution stirred for 1 h. A solution of the ketone **21** (100 mg, 0.23 mmol) in 2 mL of THF was introduced and the mixture heated at 75–80 °C for 14 h. After the usual workup and purification by TLC (silica gel, 9:1 hexane/ethyl acetate) and HPLC (Altex-ultrasphere, 50 mmol per liter silver nitrate in methanol[13] as eluent) the olefins **22** and **23** were obtained in 46% yield.

22: NMR δ 0.710 (3 H, s, 18-CH_3), 1.022 (3 H, s, 19-CH_3), 1.011 (3 H, d, J = 6.83 Hz, 21-CH_3),[18] 0.789 (3 H, d, J = 6.39 Hz, 26-CH_3), 0.767 (3 H, d, J = 6.63 Hz, 27-CH_3), 1.030 (3 H, d, J = 5.28 Hz, 29-CH_3), 3.323 (3 H, s, 6-OCH_3), 4.657 and 4.768 (2 H, 2 s, 28-CH_2); mass spectrum, m/z (relative intensity) 426 (8, M), 411 (21), 371 (29), 342 (19), 314 (30), 285 (47), 283 (32), 253 (100), 227 (33), 213 (40).

23: NMR δ 0.765 (3 H, s, 18-CH_3), 1.028 (3 H, s, 19-CH_3), 0.636 (3 H, d, J = 6.81 Hz, 29-CH_3), 0.918 (3 H, d, J = 6.11 Hz, 21-CH_3),[18] 0.884 (3 H, d, J = 6.97 Hz, 26-CH_3), 0.838 (3 H, d, J = 5.96 Hz, 27-CH_3), 3.330 (3 H, s, 6-OCH_3), 4.707 and 4.782 (2 H, 2 s, 28-CH_2); mass spectrum, m/z (relative intensity) 411 (4), 371 (11) 314 (100), 299 (30), 283 (96), 282 (93), 267 (28), 259 (38), 256 (18), 253 (44), 227 (24), 213 (28).

(22*S*)-22,23-Dimethylcholesterol (24 and 25) (Table I). A solution of 15 mg (0.035 mmol) of the olefin **23** in 5 mL of ethyl acetate was hydrogenated with PtO_2 at room temperature for 14 h. After removal of the catalyst, the solvent was evaporated in vacuo and the mixture separated by HPLC on an Altex-ultrasphere column with methanol as eluent. The *i*-methyl ether protecting group was removed from each fraction by hydrolysis with *p*-toluenesulfonic acid in 20% aqueous dioxane to afford **24** and **25**.[16]

(18) The assignments for the methyl doublets are tentative.

24: high-resolution mass spectrum, m/z (relative intensity, assignment) 414.3859 (24, $C_{29}H_{50}O$, M), 399.3636 (6, $C_{28}H_{47}O$), 396.3745 (17, $C_{29}H_{48}$), 329.3176 (9, $C_{24}H_{41}$), 255.2115 (11, $C_{19}H_{27}$), 213.1637 (11, $C_{16}H_{21}$).

25: 414.3874 (27, $C_{29}H_{50}O$, M), 399.3644 (7, $C_{28}H_{47}O$), 396.3758 (15, $C_{29}H_{48}$), 329.3206 (13, $C_{24}H_{41}$), 255.2095 (14, $C_{19}H_{27}$), 213.1646 (14, $C_{16}H_{21}$).

Hydroboration of (22*S*)-6β-Methoxy-22-methyl-23-methylene-3α,5-cyclo-5α-cholestane (22). A solution of 30 mg (0.07 mmol) of **22** in 5 mL of THF was cooled in an ice bath under nitrogen and 2 mL of an approximately 1 M solution of diborane in THF was added with stirring. The mixture was stirred for 1 h in the ice bath and then at room temperature overnight. The mixture was cooled again and 1 mL of water added dropwise followed by 2 mL of 3 N sodium hydroxide and finally by the slow addition of 2 mL of 30% hydrogen peroxide. The mixture was stirred at room temperature for 3 h and then extracted with dichloromethane (3 × 5 mL). The combined organic extracts were washed with water and saturated sodium chloride and dried over sodium sulfate. After evaporation of the solvent the two epimeric alcohols **26** and **27** were separated by TLC (silica gel, 8:2 hexane/ethyl acetate as eluent).

Less polar alcohol 27: NMR δ 0.683 (3 H, s, 18-CH_3), 0.749 (3 H, d, J = 6.96 Hz, 29-CH_3), 0.840 (3 H, d, J = 6.51 Hz, 27-CH_3), 0.858 (3 H, d, J = 6.66 Hz, 26-CH_3), 0.929 (3 H, d, J = 6.90 Hz, 21-CH_3), 1.020 (3 H, s, 19-CH_3), 3.321 (3 H, s, 6-OCH_3), 3.463–3.692 (2 H, m, 28-CH_2OH).

More polar alcohol 26: NMR δ 0.677 (3 H, s, 18-CH_3), 0.780 (3 H, d, J = 7.07 Hz, 29-CH_3), 0.832 (3 H, d, J = 6.69 Hz, 27-CH_3), 0.847 (3 H, d, J = 6.98 Hz, 26-CH_3), 0.951 (3 H, d, J = 7.05 Hz, 21-CH_3), 1.024 (3 H, s, 19-CH_3), 3.322 (3 H, s, 6-OCH_3), 3.438–3.671 (2 H, m, 28-CH_2OH).

(22*R*)-22,23-Dimethylcholesterol (28 and 29). (Table I). A solution of 10 mg (0.023 mmol) of alcohol **26** in 0.5 mL of dry dichloromethane containing 0.04 mL (0.29 mmol) of triethylamine was cooled in an ice bath and 0.012 mL (0.17 mmol) of methanesulfonyl chloride was added with stirring. After 30 min the solvent was removed in vacuo to give the crude mesylate which was immediately taken up in 5 mL of dry THF. Excess lithium aluminum hydride was added and the mixture refluxed overnight. After the usual workup, the residue was purified by HPLC (Altex-ultrasphere, 9:1 methanol/ethyl acetate as eluent) and the *i*-methyl ether protecting group removed by hydrolysis with *p*-toluenesulfonic in 20% aqueous dioxane to afford **28**. Similar treatment of alcohol **27** gave **29**. The mass spectra of **28** and **29** were identical: m/z (relative intensity) 414 (100, M), 399 (15), 396 (29), 329 (33), 303 (15), 273 (23), 255 (31), 241 (15), 231 (38), 228 (17), 219 (15), 215 (24), 213 (72).

General Procedure for the Introduction of Deuterium. The mesylate prepared from alcohol **26** (5 mg, 0.012 mmol) (see above) was reduced with lithium aluminum deuteride in THF. After the usual workup and purification by HPLC (Altex-ultrasphere, 9:1 methanol/ethyl acetate as eluent) the *i*-methyl ether protecting group was removed by hydrolysis with *p*-toluenesulfonic acid in 20% aqueous dioxane to afford the C-28 monodeuterated (22*R*)-22,23-dimethylcholesterol (**28**).

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Registry No. **15**, 25819-77-6; **17**, 93756-15-1; **18**, 28322-40-9; (*E*)-**19**, 93756-16-2; (*Z*)-**19**, 93781-68-1; **21** isomer 1, 93756-17-3; **21** isomer 2, 93756-18-4; **22**, 93756-19-5; **23**, 93756-20-8; **24**, 93756-21-9; **25**, 93756-22-0; **26**, 93756-23-1; **27**, 93781-76-1; **28**, 93756-24-2; **29**, 93756-25-3; CH_3I, 74-88-4; Ph_3PCH_3Br, 1779-49-3.