were filtered off, there was obtained a viscous oil, which TLC indicated was a mixture of five compounds. Careful chromatography on silica gel and elution with chloroform–acetone (7:3) gave 0.005 g (19%) of 4-epiisocelorbicol (**26**) as a white solid. Recrystallization from acetone afforded crystals: mp 148–150 °C; NMR δ 0.91 (d, J = 6.6 Hz, CH_3CH), 1.10 (s, 3 H, CH_3), 1.21, 1.53 (s, 3 H each, $(CH_3)_2C$), 2.89 (d, J = 10.0 Hz, 9-OH), 3.53 (m, 1 H, 9α-H), 4.14 (br s, 2 H, 1β,2β-H); MS, m/e (relative intensity) 270 (35), 255 (46), 252 (17), 237 (31), 219 (42), 208 (17), 201 (15), 191 (13), 183 (14), 168 (46).

(±)-Isocelorbicol (1). A solution of 0.051 g (0.11 mmol) of hydroxy acetate **25** was hydrogenated as described above and the product hydrolyzed at room temperature by using 6 mL of 0.2 M barium hydroxide as described by Smith et al.[3] Chromatography on silica gel gave first 0.0049 g (17%) of 4-epiisocelorbicol (**26**) identical with that described above and then 0.0035 g (12%) of an approximately 2:1 mixture of (±)-isocelorbicol (**1**) and triol **26**: NMR δ 1.21 (s, 6 H, CH_3), 1.25 (d, J = 7.2 Hz, CH_3CH), 1.48 (s, 3 H, CH_3), 3.2 (m, 1 H, 9α-H), 4.16 (m, 2 H, 1β-H, 2β-H); MS, m/e (relative intensity) 270 (30), 255 (31), 252 (18), 237 (26), 219 (31), 208 (18), 183 (11), 168 (51), 154 (15), 151 (27), 137 (50). Careful rechromatography of 0.0062 g of the 2:1 mixture of triols **1** and **26** failed to effect separation.

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Registry No. (±)-**1**, 88764-00-5; (±)-**9**, 88764-01-6; (±)-**12**, 88703-19-9; (±)-**13**, 88703-20-2; (±)-**15**, 88703-21-3; (±)-**15** (1β-benzoate), 88703-22-4; (±)-**16**, 88703-23-5; (±)-**16** (acetonide), 88703-24-6; (±)-**17**, 88703-25-7; (±)-**18**, 88703-26-8; (±)-**19**, 88703-27-9; (±)-**20**, 88764-02-7; (±)-**21**, 88764-03-8; (±)-**22**, 88703-28-0; (±)-**24**, 88703-29-1; (±)-**25**, 88764-04-9; (±)-**26**, 88764-05-0.

Synthesis and Stereochemical Assignment of (22*R*,24*S*)-, (22*R*,24*R*)-, (22*S*,24*R*)-, and (22*S*,24*S*)-22,24-Dimethylcholesterol

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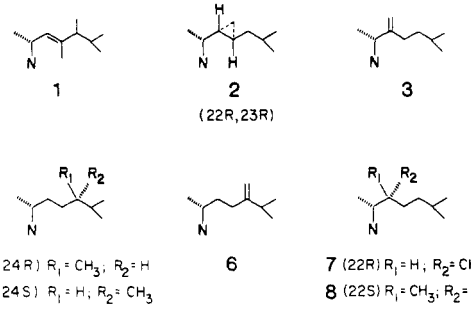
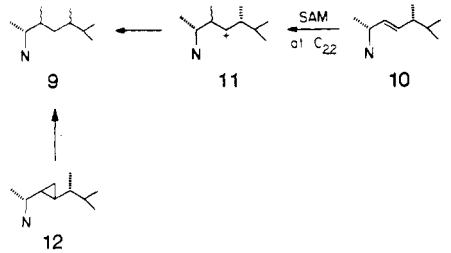
22,24-Dimethylcholesterol, though hitherto unknown, is likely to exist in nature. In order to expedite its recognition, all four isomers have been synthesized and their stereochemistry established. Selective deuterium labeling was used for the assignment of the NMR shifts of the methyl signals at C-22 and C-24.

The existence in the marine environment of 23,24-dimethylcholesterols (e.g., **1**) is well documented.[1-3] The recent isolation from marine organisms of (22*R*,23*R*)-22,23-methylenecholesterol (**2**)[4] and 22-methylenecholesterol (**3**)[5] demonstrates that direct bioalkylation of 22-dehydrocholesterol is possible in nature. Since (24*R*)-24-methylcholesterol (**4**) and its 24*S* epimer (**5**) coexist with 24-methylenecholesterol (**6**)[6] it is likely that (22*R*)-22-methylcholesterol (**7**) and its 22*S* epimer (**8**) may also be naturally occurring. Similarly, it is conceivable that 22,24-dimethylcholesterol (**9**) could also arise in nature by





attack of *S*-adenosylmethionine (SAM) at C-22 of brassicasterol (**10**) followed by loss of a C-22 or C-24 proton from the carbonium ion **11**[7] and subsequent biohydrogenation. Alternatively, enzymatic isomerization of demethylgorgosterol (**12**) followed by biohydrogenation could also lead to such a compound.[8]

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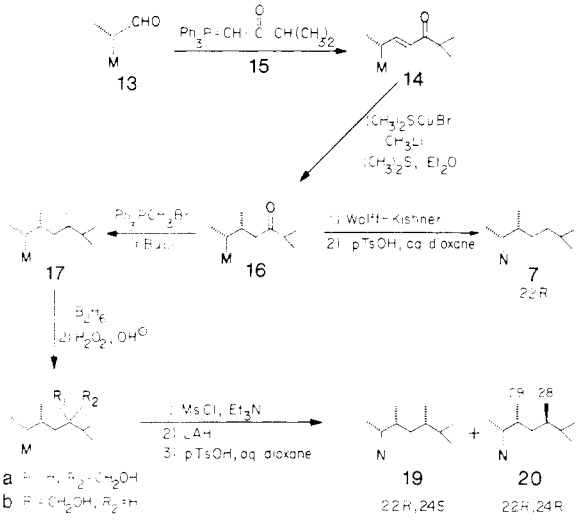
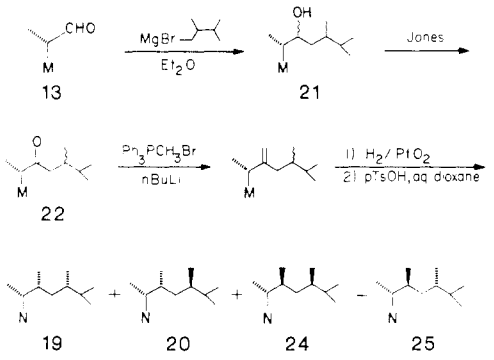
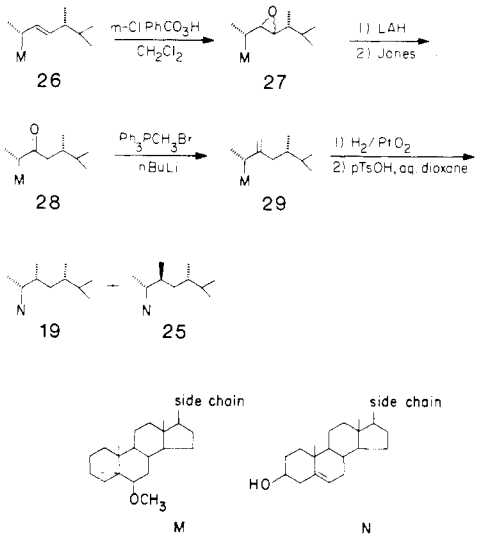
Scheme I



Scheme II



Scheme III



To expedite their eventual isolation from marine sources, we have undertaken the synthesis of all four stereoisomers of 22,24-dimethylcholesterol in order to provide authentic reference compounds of known configuration and to indicate which physical method would be of greatest diagnostic utility.

The synthetic schemes (Schemes I–III) that were followed allowed for a definitive determination of the absolute configuration at both the C-22 and C-24 positions. In Scheme I the synthesis started with the aldehyde **13** which was converted to the known dienone **14**[9] by Wittig reaction with the phosphorane **15**. 1,4-Addition of dimethylcopper lithium[10] to this unsaturated ketone **14** furnished predominantly the C-22 *R* epimer **16** as shown by its NMR spectrum and by the removal of the C-24 ketone functionality to give the known (22*R*)-22-methylcholesterol (**7**).[11]

Wittig reaction of the ketone **16** with methyltriphenylphosphonium bromide gave the C-24 methylene compound **17** which on hydroboration yielded the epimeric alcohols **18a** and **18b**. These were separated by HPLC and each pure alcohol was then transformed via lithium aluminum hydride reduction of its mesylate and removal of the protecting group to pure (22*R*,24*S*)-22,24-dimethylcholesterol (**19**) and its 24*R* epimer (**20**) (vide infra).

In Scheme II the aldehyde **13** was converted to the alcohol **21** by the addition of the Grignard reagent prepared from 2,3-dimethylbromobutane. Jones oxidation of **21** gave the ketone **22** which was transformed to the C-22 methylene compound **23** with methyltriphenylphosphonium bromide. Hydrogenation of this olefin **23** using platinum oxide followed by HPLC separation and removal of the *i*-methyl ether protecting group furnished the four stereoisomers **19**, **20**, **24**, and **25**, the former two having been already prepared via Scheme I and their C-22(*R*) stereochemistry established; the latter two, viz., **24** and **25**, must therefore possess the same C-22(*S*) configuration and differ only at C-24.

The stereochemistry at C-24 was determined by following the synthetic Scheme III in which the *i*-methyl ether of brassicasterol (**26**) (obtained by lithium–ethylamine reduction of the Diels–Alder adduct of ergosterol and 4-phenyl-1,2,4-triazoline-3,5-dione[12]) was converted to the epoxide **27** with metachloroperbenzoic acid. Lithium aluminum hydride reduction of **27** gave a mixture of the C-22 and C-23 alcohols which were oxidized with Jones reagent to the corresponding ketones; HPLC separation gave the desired C-22 ketone **28** which was then transformed to the 22,24-dimethylcholesterols **19** and **25** following the procedure outlined above for the ketone **22**. Since one of these sterols (**19**) possesses an NMR spectrum identical with one of the sterols (**19**) obtained in Scheme I (and **19** in Scheme II) and since the C-22 *R* stereochemistry in **19** had already been established (vide supra), **19** must possess the 22*R*,24*S* configuration. Therefore **20**, being different from **19** only by the stereochemistry at C-24, must have the 22*R*,24*R* configuration. The other sterol, **25**, obtained from brassicasterol (and **25** from Scheme II), being different from **19** only by the stereochemistry at C-22, must therefore possess the 22*S*,24*S* configuration. It follows that the only remaining stereoisomer, **24**, must have the 22*S*,24*R* stereochemistry.

Table I summarizes the proton and ^{13}C NMR data of the four 22,24-dimethylcholesterols. The assignment of the C-28 and C-29 methyl signals was based on the selective syntheses of the monodeuterated compounds. The C-22 epimeric ketones **30** were converted to the C-24

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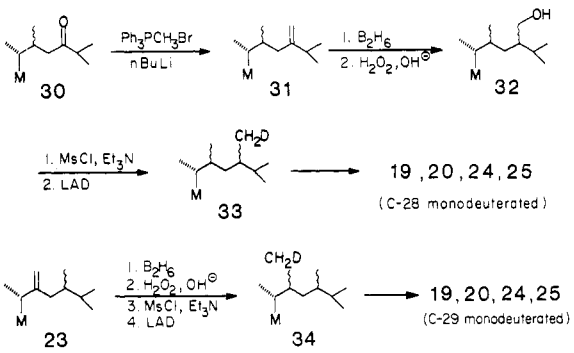
Table I. Proton (300-MHz) and ^{13}C NMR Spectral Data[a] of Isomeric 22,24-Dimethylcholesterols 19, 20, 24, and 25

	(22R,24S)-22,24-dimethylcholesterol (19)		(22R,24R)-22,24-dimethylcholesterol (20)		(22S,24R)-22,24-dimethylcholesterol (24)		(22S,24S)-22,24-dimethylcholesterol (25)	
carbon	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR
1	37.23		37.24		37.21		37.24	
2	31.62		31.63		31.59		31.64	
3	71.75		71.76		71.74		71.74	
4	42.27		42.27		42.22		42.27	
5	140.71		140.73		140.71		140.74	
6	120.69		121.71		121.66		121.69	
7	31.86		31.87		31.87		31.88	
8	31.88		31.91		31.91		31.91	
9	50.10		50.13		50.12		50.14	
10	36.47		36.48		36.47		36.47	
11	21.08		21.10		21.10		21.11	
12	39.81		39.84		39.87		39.91	
13	42.27		42.27		42.22		42.27	
14	56.70		56.69		56.79		56.76	
15	24.34		24.31		24.20		24.23	
16	28.15		27.85		27.85		27.80	
17	53.50		53.41		53.75		54.02	
18	11.68	0.676	11.80	0.680	11.78	0.686	11.70	0.683
19	19.37	1.006	19.40	1.008	19.38	1.011	19.37	1.009
20	31.78		33.78		32.10		32.25	
21	12.98	0.790 (d, J = 6.02 Hz)	12.85	0.785 (d, J = 6.54 Hz)	12.09	0.743 (d, J = 6.44 Hz)	12.71	0.774 (d, J = 6.22 Hz)
22	41.92		41.68		35.17		40.77	
23	34.21		33.18		39.50		40.13	
24	35.70		35.70		36.92		35.80	
25	28.87		31.47		31.12		31.59	
26	19.37	0.876 (d, J = 6.91 Hz)	19.84	0.845 (d, J = 6.86 Hz)	20.12	0.853 (d, J = 6.84 Hz)	17.80	0.849 (d, J = 6.82 Hz)
27	21.38	0.736 (d, J = 6.67 Hz)	18.79	0.819 (d, J = 6.83 Hz)	17.91	0.792 (d, J = 7.01 Hz)	20.18	0.787 (d, J = 6.81 Hz)
28	15.33	0.754 (d, J = 6.57 Hz)	15.39	0.755 (d, J = 6.66 Hz)	15.56	0.765 (d, J = 6.68 Hz)	15.44	0.757 (d, J = 6.59 Hz)
29	15.67	0.844 (d, J = 6.86 Hz)	18.79	0.819 (d, J = 6.83 Hz)	14.04	0.688 (d, J = 6.58 Hz)	12.51	0.658 (d, J = 6.80 Hz)

[a] The ^{13}C NMR data are for the carbons indicated and the ^1H NMR data for the corresponding hydrogens on those carbons.

Scheme IV



methylene compounds **31** (Scheme IV). Hydroboration of the latter gave a mixture of the alcohols **32** whose mesylates were reduced with lithium aluminum deuteride to give a mixture of the *i*-methyl ethers of C-28 monodeuterated 22,24-dimethylcholesterols **33**. HPLC separation and removal of the *i*-methyl ether protecting group furnished the pure C-28 monodeuterated 22,24-dimethylcholesterols **19**, **20**, **24**, and **25**. Similar treatment of the C-22 methylene compounds **23** furnished the C-29 monodeuterated 22,24-dimethylcholesterols. The ^1H NMR spectra of these deuterated sterols (see Figure 1) showed a considerable reduction in size and broadening of the signal of the deuterated methyl group, enabling unambiguous assignment of the C-28 and C-29 methyl signals. The broad band decoupled ^{13}C spectra showed triplets for the deuterated methyls.

Table II. Physical Constants of 22,24-Dimethylcholesterols

sterol	mp, °C	GC relative t_r[a] (3% OV-17)	HPLC relative t_r[a] (ODS-2)
(22R,24S)-**19**	161-162	1.34	0.80
(22R,24R)-**20**	182-183	1.43	0.82
(22S,24R)-**24**	145-146	1.50	0.93
(22S,24S)-**25**	176-177	1.56	1.00

[a] Cholesterol t_r = 1.00.

As was observed for the monomethylcholesterols[11] the C-29(*S*) isomers **24** and **25** show a large upfield shift for the C-29 methyl signal. 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 properties will greatly facilitate the search for such compounds from natural marine sources.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and

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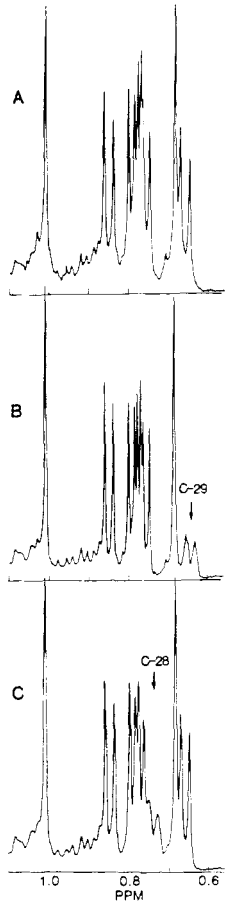

Figure 1. ^{1}H NMR of the methyl region of (22*S*,24*S*)-22,24-dimethylcholesterol (**25**) (A), C-29 monodeuterated (B), and C-28 monodeuterated (C).

are uncorrected. Gas chromatography was performed on a U-shaped column packed with 3% OV-17 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 and ^{13}C NMR spectra were recorded on a Nicolet NMC 300-MHz wide-bore spectrometer with $CDCl_3$ as solvent. Chemical shifts are given in parts per million and J values in hertz. High-resolution spectral data were obtained on a Finnigan Mat 711 Spectrometer and combined GC/MS was performed on a Finnigan Mat 44 instrument.

(22*R*)-6β-Methoxy-3α,5-cyclo-22-methyl-5α-cholestan-24-one (16). Cuprous bromide–methyl sulfide complex (from Fluka AG, Switzerland, 340 mg, 1.66 mmol) was dissolved in 2 mL of methyl sulfide and 2 mL of anhydrous ether; 2 mL of 1.55 M methyllithium (3.10 mmol) in ether was added dropwise. (22*E*)-6β-Methoxy-3α,5-cyclo-5α-cholest-22-en-24-one (**14**) (500 mg 1.21 mmol) in 10 mL of ether was added and the mixture was stirred under argon at room temperature for 2 h. An aqueous solution of ammonium chloride/ammonia (pH 8) was added and the mixture partitioned between the aqueous solution and ether. The aqueous phase was extracted with ether and the combined ether extracts were washed with water, saturated brine, and dried over sodium sulfate. Evaporation gave 500 mg (97%) of an oil (**16**) which was purified by preparative thin-layer chromatography on silica gel plates with 9:1 hexane/ethyl acetate as eluent: NMR δ 0.630 (3 H, s, 18-CH_3), 0.715 (3 H, d, J = 6.82 Hz, 28-CH_3), 0.741 (3 H, d, J = 6.61 Hz, 21-CH_3), 0.921 (3 H, s, 19-CH_3), 0.979 (6 H, d, J = 6.92 Hz, 26- and 27-CH_3), 3.219 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 428 (49, M), 413 (39), 396 (58), 373 (81), 342 (76), 300 (72), 284 (100), 255 (87).

(22*R*)-6β-Methoxy-3α,5-cyclo-22-methyl-24-methylene-5α-cholestane (17). Methyltriphenylphosphonium bromide (2.13, 6 mmol) in dry benzene (10 mL) was treated with n-BuLi (2.5 mL of a 2.4 N solution in hexane, 6 mmol). After 1 h of reflux, a solution of the ketone **16** (500 mg, 1.17 mmol in 5 mL benzene) was added and the reaction mixture refluxed for 48 h. After the usual workup, the 24-methylene compound **17** was obtained in 60% yield after preparative thin-layer chromatography on silia gel plates with 9:1 hexane/ethyl acetate as eluent: NMR δ 0.629 (3 H, s, 18-CH_3), 0.694 (3 H, d, J = 7.25 Hz, 21-CH_3), 0.714 (3 H, d, J = 6.93 Hz, 29-CH_3), 0.913 (3 H, d, J = 6.80 Hz, 26-CH_3), 0.922 (3 H, s, 19-CH_3), 0.933 (3 H, d, J = 7.45 Hz, 27-CH_3), 3.224 (3 H, s, 6-OCH_3), 4.551 and 4.648 (2 H, 2 s, 28-CH_2); mass spectrum, m/z (relative intensity) 426 (19, M), 411 (55), 394 (30), 371 (100), 342 (75), 304 (98), 300 (98).

Hydroboration of (22*R*)-6β-Methoxy-3α,5-cyclo-22-methyl-24-methylene-5α-cholestane (17). A solution of 300 mg (0.7 mmol) of **17** in 15 mL of tetrahydrofuran was cooled in an ice bath under nitrogen, and 20 mL of an approximately 1 M solution of diborane in THF was added with stirring. The mixture was stirred for 1 h in an ice bath and then at room temperature overnight. The mixture was cooled again, and 15 mL of water was added dropwise, followed by 15 mL of 3 N sodium hydroxide, and finally by the slow addition of 15 mL of 30% hydrogen peroxide. The mixture was stirred at room temperature for 2 h and then extracted with chloroform (3 × 50 mL). The combined chloroform extracts were washed successively with water and saturated sodium chloride solution and dried over magnesium sulfate. The two epimeric alcohols **18a** and **18b** were separated by HPLC (Altex-ultrasphere, 95:5 methanol/water as eluent) and the combined yield was 90%.

18a: NMR δ 0.717 (3 H, s, 18-CH_3), 0.806 (3 H, d, J = 6.38 Hz, 21-CH_3), 0.838 (3 H, d, J = 7.05 Hz, 26-CH_3), 0.864 (3 H, d, J = 6.85 Hz, 29-CH_3), 0.949 (3 H, d, J = 6.97 Hz, 27-CH_3), 1.014 (3 H, s, 19-CH_3), 3.318 (3 H, s, 6-OCH_3), 3.68–3.75 (2 H, m, 28-CH_2OH).

18b: NMR δ 0.720 (3 H, s, 18-CH_3), 0.797 (3 H, d, J = 6.67 Hz, 21-CH_3), 0.845 (3 H, d, J = 6.99 Hz, 26-CH_3), 0.865 (3 H, d, J = 6.87 Hz, 29-CH_3), 0.931 (3 H, d, J = 6.86 Hz, 27-CH_3), 1.019 (3 H, s, 19-CH_3), 3.322 (3 H, s, 6-OCH_3), 3.62–3.66 (2 H, m, CH_2OH).

The mass spectra of **18a** and **18b** were identical, m/z (relative intensity) 444 (100), 329 (52), 412 (98), 389 (97), 385 (31), 273 (40), 255 (71).

(22*R*,24*S*)-22,24-Dimethylcholesterol (19 in Table I). A solution of 123 mg (0.28 mmol) of alcohol **18a** in 1 mL dry methylene chloride containing 0.078 mL (0.56 mmol) of triethylamine was cooled in an ice bath and 0.026 mL (0.33 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 15 mL of dry THF. Excess lithium aluminum hydride was added and the mixture was refluxed overnight. After the usual workup the residue was purified by preparative TLC (silica gel, 9:1 hexane/ethyl acetate as eluent) and the i-methyl ether protecting group removed by hydrolysis with p-toluenesulfonic acid in 10% aqueous dioxane to afford **19**: high-resolution mass spectrum, m/z (relative intensity, assignment) 414.38572 (100, $C_{29}H_{50}O$, M), 399.36160 (13.03, $C_{28}H_{47}O$), 396.37406 (31.48, $C_{29}H_{48}$), 329.32173 (15.41, $C_{24}H_{41}$), 231.17526 (12.06, $C_{16}H_{23}O$), 213.16229 (21.69, $C_{16}H_{21}$).

(22*R*,24*R*)-22,24-Dimethylcholesterol (20 in Table I). The alcohol **18b** was treated exactly as described above to give **20**: high-resolution mass spectrum, m/z (relative intensity, assignment) 414.38792 (100, $C_{29}H_{50}O$, M), 399.36378 (11.06, $C_{28}H_{47}O$), 396.37524 (22.54, $C_{29}H_{48}$), 329.31881 (12.77, $C_{24}H_{41}$), 255.20906 (13.23, $C_{19}H_{27}$), 213.16257 (13.81, $C_{16}H_{21}$).

6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholestan-22-ol (21). The aldehyde **13** (1.75 g, 5.1 mmol) in 15 mL of ether was added slowly to the Grignard solution from 1.2 g (50 mmol) of magnesium and 6 g (36 mmol) of 2,3-dimethyl-1-bromobutane (from 2,3-dimethylbutan-1-ol and PBr_3) in ether. The mixture was refluxed overnight and worked up in the usual manner to give the crude alcohol **21** which was purified by preparative TLC (silica gel, 7:3 hexane/ethyl acetate as eluent): mass spectrum, m/z (relative intensity) 430 (90, M), 415 (55), 398 (100), 375 (92), 372 (38), 301 (20), 284 (53), 255 (28).

6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholestan-22-one (22). A solution of 1.5 g (3.45 mmol) of the alcohol **21** in 75 mL of

acetone was 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 **22** in 53% yield: NMR δ 0.735 (3 H, s, 18-CH_3), 0.790, 0.804 (3 H, 2 d, J = 6.77, 7.33 Hz, 26-CH_3), 0.822, 0.824 (3 H, 2 d, J = 5.74, 6.99 Hz, 27-CH_3), 0.864 (3 H, d, J = 6.83 Hz, 28-CH_3), 1.020 (3 H, s, 19-CH_3), 1.075 (3 H, d, J = 6.87 Hz, 21-CH_3), 3.321 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 428 (83, M), 413 (50), 396 (91), 373 (100), 370 (25), 326 (23), 283 (41), 255 (48).

6β-Methoxy-3α,5-cyclo-24-methyl-22-methylene-5α-cholestane (23). Methyltriphenylphosphonium bromide (2.1 gm, 6 mmol) in dry benzene (10 mL) was treated with n-BuLi (2 mL of a 2.4 M solution in hexane, 4.8 mmol). After 1 h of reflux, a solution of the ketone **22** (420 mg, 0.98 mmol in 5 mL benzene) was added and the reaction mixture refluxed for 40 h. After the usual workup, the 22-methylene compound was obtained in 34% yield after preparative thin-layer chromatography (silica gel plates with 95:5 hexane/ethyl acetate as eluent) followed by HPLC (Whatman Partisil M20 10/50 column with methanol as eluent): NMR δ 0.736 (3 H, s, 18-CH_3), 0.785 (3 H, d, J = 6.36 Hz, 26-CH_3), 0.818 (3 H, d, J = 6.72 Hz, 27-CH_3), 0.881 (3 H, d, J = 6.58 Hz, 28-CH_3), 1.021 (3 H, d, J = 6.11 Hz, 21-CH_3), 1.026 (3 H, s, 19-CH_3), 3.323 (3 H, s, 6-OCH_3), 4.621 and 4.758 (2 H, 2 s, 29-CH_2); mass spectrum, m/z (relative intensity) 426 (19, M), 411 (26), 394 (15), 371 (59), 356 (100), 341 (26), 324 (99), 301 (34), 298 (39), 271 (14), 255 (83).

22,24-Dimethylcholesterols (19, 20, 24, and 25 in Table I). A solution of 280 mg (0.66 mmol) of the olefin **23** in 10 mL of ethyl acetate was hydrogenated with 50 mg of PtO_2 at room temperature for 14 h. After removal of the catalyst, the solvent was evaporated under reduced pressure. The mixture was separated on an Altex-ultrasphere column with methanol as eluent. Each fraction so obtained was then deprotected in the usual way to give **19**, **20**, **24**, and **25**: for NMR data see Table I; all had identical mass spectra, m/z (relative intensity) 414 (100, M), 399 (13), 396 (31), 381 (8), 329 (15), 303 (10), 255 (8), 231 (12), 213 (21).

(24*S*)-6β-Methoxy-3α,5-cyclo-22,23-epoxy-24-methyl-5α-cholestane (27). m-Chloroperbenzoic acid (150 mg, 0.87 mmol) was added to the i-methyl ether of brassicasterol **26** (267 mg, 0.65 mmol) in dichloromethane (10 mL) and the mixture was kept at room temperature for 24 h. The mixture was then filtered through Woelm neutral alumina[14] and the filtrate concentrated. Preparative thin-layer chromatography (silica gel with 9:1 hexane/ethyl acetate as eluent) gave the two epimeric epoxides **27** in 79% yield.

Less Polar Epoxide: NMR δ 0.702 (3 H, s, 18-CH_3), 0.913 (3 H, d, J = 6.95 Hz, 26-CH_3), 0.920 (3 H, d, J = 6.82 Hz, 27-CH_3), 0.963 (3 H, d, J = 6.79 Hz, 28-CH_3), 1.022 (3 H, s, 19-CH_3), 1.084 (3 H, d, J = 6.04 Hz, 21-CH_3), 2.36–2.69 (2 H, m, 22- and 23-CH), 3.331 (3 H, s, 6-OCH_3); mass spectrum, m/z (relative intensity) 428 (80, M), 413 (54), 396 (76), 373 (100), 370 (26), 312 (12), 289 (15), 255 (32), 253 (40).

More Polar Epoxide: NMR δ 0.710 (3 H, s, 18-CH_3), 0.925 (3 H, d, J = 6.67 Hz, 26-CH_3), 0.948 (3 H, d, J = 6.69 Hz, 27-CH_3), 0.978 (3 H, d, J = 5.95 Hz, 28-CH_3), 1.084 (3 H, d, J = 6.38 Hz, 21-CH_3), 2.43–2.60 (2 H, m, 22- and 23-CH), 3.316 (3 H, s, 6-OCH_3). The mass spectrum was indistinguishable from that of the less polar epoxide.

(24*S*)-6β-Methoxy-3α,5-cyclo-24-methyl-5α-cholestan-22-one (28). The epoxide mixture **27** (219 mg, 0.51 mmol) and lithium aluminum hydride (0.4 g, 11 mmol) in tetrahydrofuran (20 mL) and ether (20 mL) was heated under reflux for 3 days. After the usual workup, the crude mixture of alcohols obtained was oxidized with Jones reagent to give the ketone **28** and its regioisomer in 30% yield from the epoxides **27**. Preparative TLC (silica gel, 9:1 hexane/ethyl acetate as eluent) and HPLC (Altex ultraspere, methanol as eluent) gave the ketone **28**: NMR δ 0.739 (3 H, s, 18-CH_3), 0.807 (3 H, d, J = 6.71 Hz, 26-CH_3), 0.823 (3 H, d, J = 6.75 Hz, 27-CH_3), 0.866 (3 H, d, J = 6.83 Hz, 28-CH_3), 1.023 (3 H, s, 19-CH_3), 1.080 (3 H, d, J = 6.82 Hz, 21-CH_3); mass spectrum, m/z (relative intensity) 428 (83, M), 413 (50), 396 (91), 373 (100), 370 (25), 326 (23), 283 (41), 255 (48).

The ketone **28** was then converted to the 22,24-dimethylcholesterols **19** and **25** via the olefin **29** by methods described above for the ketone **16**.

General Procedure for the Introduction of Deuterium. The mesylates prepared from olefin **23** (61 mg, 0.14 mmol) (see above) were reduced with excess lithium aluminum deuteride in THF to give the C-29 monodeuterated i-methyl ethers of 22,24-dimethylcholesterols (**34**). These were fractionated by HPLC (Altex ultrasphere column, methanol as eluent) to give the four stereoisomers. The protecting group of each stereoisomer was removed by hydrolysis with p-toluenesulfonic acid in aqueous dioxane to afford the C-29-monodeuterated 22,24-dimethylcholesterols **19**, **20**, **24**, and **25**.

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