product showed a multiplet present at δ 4.60–4.25, identical with that of **14b**.[7] As in the case of hydrogenation of **9a** a less polar product (R_f 0.46), presumably 13,14-dihydro-15-oxo-PGI$_1$, was formed as a minor product.

Registry No. 3, 37517-42-3; **4a**, 61604-78-2; **4b**, 61600-83-7; **5a**, 80186-31-8; **5b**, 80186-32-9; **6a**, 80186-33-0; **6b**, 80186-34-1; **7a**, 80186-35-2; **7b**, 80186-36-3; **8a**, 80186-37-4; **8b**, 80186-38-5; **9a**, 80186-39-6; **9a** methyl ester, 80186-40-9; **9a** TMS derivative, 80206-12-8; **9b**, 80186-41-0; **9b** TMS derivative, 80186-42-1; **10a**, 80206-13-9; **10b**, 80206-14-0; **11a**, 80206-15-1; **11b**, 80206-16-2; **12a**, 80227-15-2; **12b**, 80227-16-3; **13a**, 62777-90-6; **14a**, 75351-76-7; **14a** TMS derivative, 80186-43-2; 4-[(dimethyl-*tert*-butylsilyl)oxy]-1-pentyne, 80186-44-3; 1-pentyn-4-ol, 2117-11-5.

Stereoselective Synthesis of (22*R*)- and (22*S*)-22-Methylcholesterol

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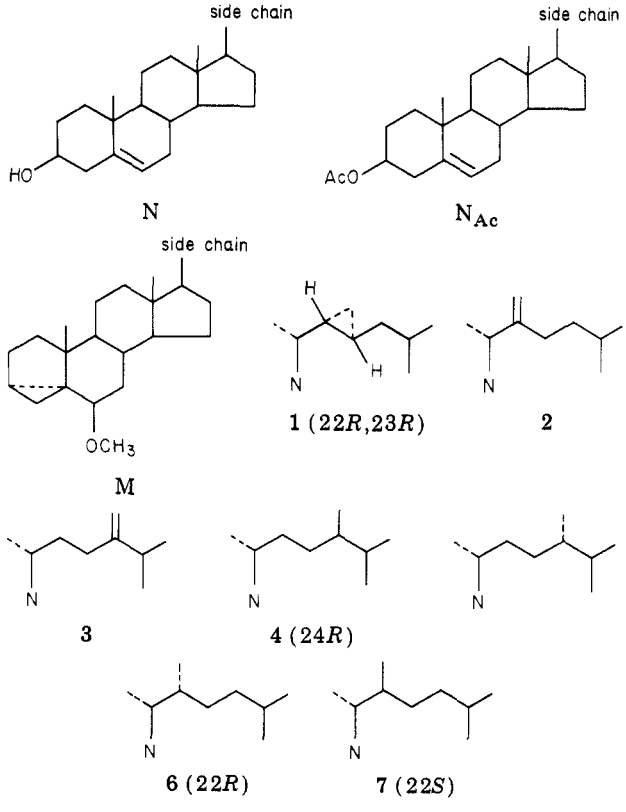
22-Methylcholesterol, though hitherto unknown, is likely to exist in nature in the marine environment. In order to facilitate its eventual recognition, stereoselective syntheses of the 22*R* and 22*S* isomers of 22-methylcholesterol were developed by using the Claisen rearrangement of appropriate precursors of established absolute configuration. An alternate approach involved hydroboration of an appropriate 22-methylene precursor, separation of the isomeric primary alcohols, mesylation, and lithium aluminum hydride reduction. Differentiation of these two isomers between themselves and from the common (24*R*)- and (24*S*)-22-methylcholesterols is possible on the basis of proton and ^{13}C NMR measurements as well as chromatographic mobility.

The recent isolation from marine organisms of (22*R*,23*R*)-22,23-methylenecholesterol (**1**)[2] and 22-methylenecholesterol (**2**)[3] (see chart I) represents the strongest indication to date that direct bioalkylation of 22-dehydrocholesterol is possible in nature. The occurrence of **1** and **2** had been predicted by us[4] and their eventual recognition was facilitated by prior synthesis of authentic reference compounds. By analogy to the existence[5] in nature of 24-methylenecholesterol (**3**) together with its two isomeric dihydro analogues, (24*R*)- (**4**) and (24*S*)-24-methylcholesterol (**5**), it is reasonable to assume that (22*R*)- (**6**) and (22*S*)-22-methylcholesterol (**7**) should also be naturally occurring. To expedite their eventual isolation from marine sources, we have undertaken their stereospecific synthesis in order to provide authentic reference compounds and to determine which physical method would be of greatest diagnostic utility.

Since knowledge of the C-22 stereochemistry is of great biosynthetic relevance, we selected a synthetic approach, which would not only be stereoselective, but would also establish the absolute configuration of the two 22-methylcholesterols (**6**, **7**). The stereospecific and regiospecific features of the Claisen rearrangement[6] have already been used to good advantage in the stereospecific introduction of certain substiuents into the steroid side chain,[7] and we have now adapted it to the synthesis of **6** and **7**.

Chart I



Our starting material, (22*E*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-one (**8**),[8] was readily available from stig-

(1) (a) Postdoctorate fellow on leave from the Technical University, Gdansk, Poland. (b) Visiting investigator on leave from Shanghai Institute of Pharmaceutical Industrial Research, Shanghai, China.

(2) Blanc, P.-A.; Djerassi, C. *J. Am. Chem. Soc.* **1980**, *102*, 7113. The absolute configurational relations (opposite absolute configuration of cyclopropane compared to those of gorgosterol and 23-demethylgorgosterol) reported in this paper are correct, but the Cahn–Ingold–Prelog notation was used incorrectly and the naturally occurring compound denoted as the 22*S*,23*S* isomer (see correction in *J. Am. Chem. Soc.* **1980**, *103*, 7036.

(3) Zielinski, J.; Li, H-t.; Milkova, T. S.; Popov, S.; Marekov, N. L.; Djerassi, C. *Tetrahedron Lett.* **1981**, 2345.

(4) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, 1815.

(5) Goad, L. J. In "Biochemical and Biophysical Perspectives in Marine Biology"; Malins, D. C., Sargent, J. R., Eds.; Academic Press: New York, 1976; 213–318.

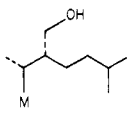
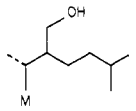
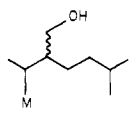
(6) Rhoads, S. J.; Raulins, N. R. *Org. React.* **1975**, *22*, 1. Ziegler, F. E. *Acc. Chem. Res.* **1977**, *10*, 227.

(7) Sucrow, W.; Slopianka, M. *Chem. Ber.* **1975**, *108*, 3721 and earlier papers. Wiersig, J. R.; Waespe-Sarcevic, N.; Djerassi, C. *J. Org. Chem.* **1979**, *44*, 3374.

(8) Anderson, G. D.; Powers, T. J., Djerassi, C.; Fayos, J.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 388.

0022-3263/82/1947-0620$01.25/0

Table I. 360-MHz NMR Data ($CDCl_3$) of Selected 22-Substituted Cholesterol Isomethyl Ethers

compd	chemical shift[a] 18-Me	19-Me	21-Me	26-Me	27-Me	28-CH_3
 17a	0.754	1.023	0.805 (J = 6.81)	0.867 (J = 6.36)	0.884 (J = 6.38)	3.600 (J = 4.25), 3.629 (J = 4.22)
 17b	0.725	1.024	0.822 (J = 6.88)	0.890 (J = 6.59)	0.898 (J = 6.55)	3.705 (J = 3.40), 3.734 (J = 2.85)
 17c	0.731	1.023	0.738 (J = 5.38)	0.894 (J = 6.79)	0.899 (J = 6.77)	3.744 (J = 3.75), 3.774 (J = 3.74)

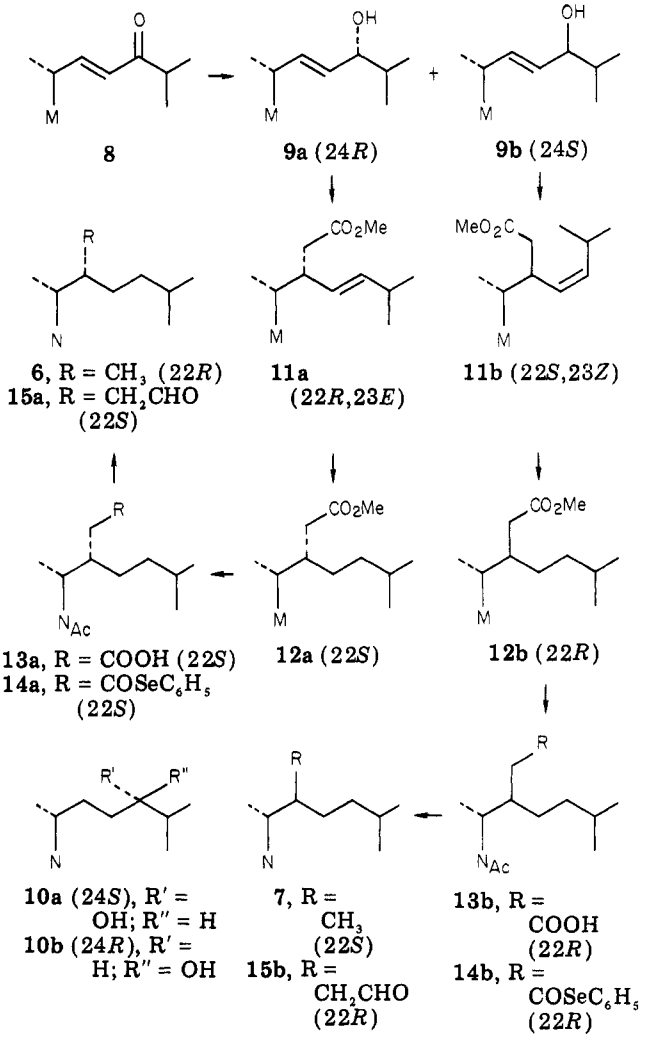
[a] Shifts are given in parts per million and J values in hertz.

masterol. Lithium aluminum hydride reduction afforded a chromatographically separable 1:1 mixture of the 24*R* and 24*S* isomers **9a** and **9b** (Scheme I), whose C-24 stereochemistry was established by hydrogenation and removal of the i-methyl ether protecting group to afford the known[9] (24*S*)- (**10a**)[10] and (24*R*)-24-hydroxycholesterols[10] (**10b**) which are readily differentiated by their characteristic ^{13}C NMR spectra.

Each allylic alcohol (**9a, b**) was separately subjected to Claisen rearrangement with trimethyl orthoacetate in refluxing xylene to provide in good yield the respective Δ^{23}-unsaturated esters **11a** and **11b**, whose C-22 stereochemistry follows from the previously established one of its 24-hydroxy precursor **9**. Catalytic hydrogenation to **12**, followed by removal of the i-methyl ether protecting group, saponification of the methyl ester function, and acetylation, provided the two cholesterol acetate isomers **13a** and **13b** with the C-22 acetic acid substituent. Decarboxylation was effected in good yield by the recently described[11] radical-induced reaction of the corresponding phenyl carboselenoates **14a** and **14b** with tributyltin hydride followed by saponification. The desired 22-methylcholesterols (**6** and **7**) were accompanied by trace quantities of epimeric **15a** and **15b**, which could be separated easily by chromatography.

As an alternate approach to 22-methylcholesterols **6** and **7** we also investigated the reduction of the double bond in the recently synthesized[3] i-methyl ether **16** of 22-methylenecholesterol (**2**). Catalytic hydrogenation with a palladium catalyst in ethyl acetate solution yielded a mixture[12] from which the pure (22*S*)-22-methylcholesterol (**7**) could be obtained in 28% yield after removal of the i-methyl ether protecting group. As shown earlier,[8] the separation of isomeric reduction products can be effected more efficiently on the products of hydroboration via the resulting primary alcohols. When this method was applied to the 22-methylenecholesterol derivatives (**16**), there was obtained a mixture of the three primary alcohols **17a–c** in a ratio of 3:96:4 (Scheme II), which could be distinguished by their characteristic proton NMR signals (Table I). Each pure isomer was transformed into the corresponding mesylate **18** and reduced with lithium aluminum hydride to **19**, and the protective group was removed to provide the pure 22-methylcholesterol isomers. The major product, **17b**, from the hydroboration was shown to possess the 22*S* configuration, since it ultimately led to (22*S*)-22-methylcholesterol (**7**), whose absolute configuration had

Scheme I



(9) Koizumi, N.; Fujimoto, Y.; Takeshita, T.; Ikekawa, N. *Chem. Pharm. Bull.* **1979**, *27*, 38.
(10) Note that the *R* and *S* notation changes when the Δ^{22} double bond is removed.
(11) Pfenninger, J.; Heuberger, C.; Graf, W. *Helv. Chim. Acta* **1980**, 2328.
(12) Double bond migration is the predominant reaction under our conditions.

Table II. Proton (360 MHz) and ^{13}C NMR Spectral Data[a] ($CDCl_3$) of Isomeric 22- (6,7) and 24-Methylcholesterols (4, 5)

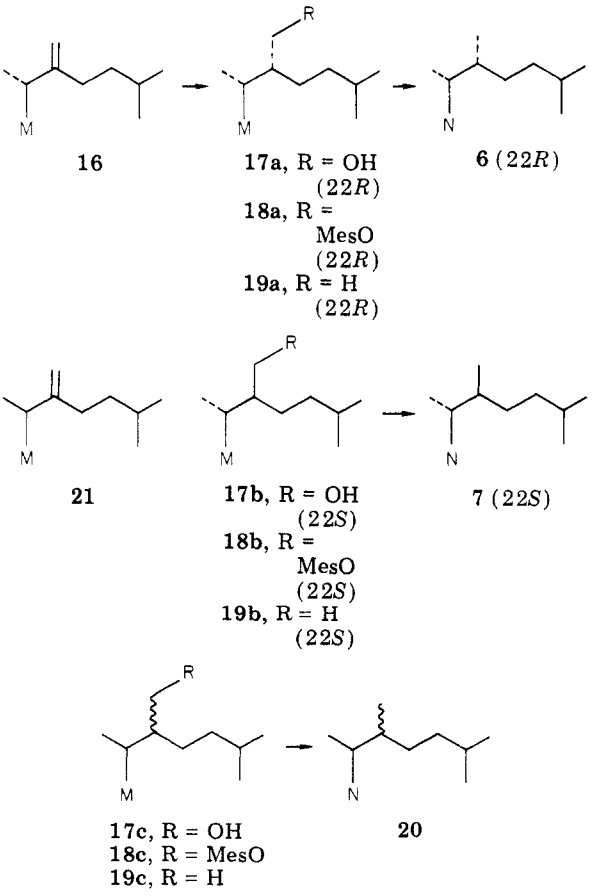
	(22R)-22-methylcholesterol (6)		(22S)-22-methylcholesterol (7)		(24R)-24-methylcholesterol (4)		(24S)-24-methylcholesterol (5)	
carbon	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
C-1	37.16		37.15		37.3		37.3	
C-2	31.55		31.57		31.7		31.7	
C-3	71.68		71.67		71.8		71.8	
C-4	42.19		42.20		42.4		42.4	
C-5	140.67		140.66		140.7		140.7	
C-6	121.58		121.56		121.7		121.7	
C-7	31.78		31.79		31.9		31.9	
C-8	31.87		31.85		31.9		31.9	
C-9	50.07		50.07		50.2		50.1	
C-10	36.40		36.39		36.5		36.5	
C-11	21.02		21.02		21.1		21.1	
C-12	39.79		39.84		39.8		39.8	
C-13	42.19		42.20		42.4		42.4	
C-14	56.64		56.71		56.8		56.8	
C-15	27.83		27.72		24.3		24.3	
C-16	24.17		24.12		28.2		28.2	
C-17	53.64		53.72		56.2		56.0	
C-18	11.70	0.675	11.70	0.690	11.9	0.680	11.9	0.678
C-19	19.28	1.008	19.27	1.013	19.4	1.007	19.4	1.009
C-20	34.80		34.59		35.9		36.1	
C-21	12.90	0.785	12.95	0.762	18.7	0.911	18.9	0.919
C-22	41.44		38.55		33.8		33.8	
C-23	26.90		33.51		30.3		30.6	
C-24	37.70		37.15		38.9		39.1	
C-25	28.33		28.22		32.4		31.5	
C-26	22.32	0.861	22.59	0.872	18.2	0.802	17.6	0.783
C-27	23.06	0.879	22.59	0.872	20.2	0.850	20.5	0.852
C-28	18.82	0.844	12.27	0.690	15.4	0.773	15.5	0.775

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

been established by the above described Claisen rearrangement sequence. One of the two minor isomers, **17a**, could be related to (22*R*)-22-methylcholesterol (**6**) and hence was the 22*R* epimer **17a**. Therefore, we assume that the third isomer, **17c**, produced in trace quantities in the hydroboration, was the C-20 isomer[13] with an unknown configuration at C-22. Whether isomerization at C-20 occurred during the hydroboration or was due to trace contamination of the starting methylene compound **16** by its C-20 isomer **21**[3] was not established. In any event, it is clear that reduction of the $\Delta^{22(28)}$ double bond is a useful route to (22*S*)-22-methylcholesterol (**7**) but not to its 22*R* isomer **6**.

In Table II we summarize the proton and ^{13}C NMR data of the two 22-methylcholesterol isomers **6** and **7** and compare their chemical shifts with those of the common, naturally occurring 24-methylcholesterol isomers **4** and **5**. Proton[14] and ^{13}C NMR[15] shifts of isomeric 24-alkylated cholesterol derivatives have already been discussed in the literature, and particular attention has been drawn to the shifts of the C-21 and C-28 methyl signals. No such studies have been performed with 22-alkyl-substituted cholesterol derivatives, since none were known until now, but data have been recorded for halo, hydroxy, and amino substituents at C-22.[16,17] Qualitatively, these results are in agreement with the data collected in Table II. Thus, Newman projections i and ii around the C-20/C-22 bonds

Scheme II

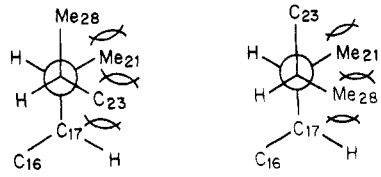


(13) According to D. M. Piatak and J. Wicha (*Chem. Rev.* **1978**, *78*, 201) the NMR chemical shift of the C-21 proton of steroids epimeric at C-20 is ca. 0.1 ppm. Since the C-21 proton chemical shift of **17c** (0.738 ppm) and its acetate (0.754 ppm) are shifted by ca. 0.08 ppm compared to those of **17b** (0.822 ppm) and its acetate (0.834 ppm), we assume that **17c** is the C-21 epimer of **17a** or **17b**.

(14) Rubinstein, I.; Goad, L. J.; Clague, A. D. H.; Mulheirn, L. J. *Phytochemistry*, **1976**, *15*, 195.

(15) Wright, J. L. C.; McInnes, A. G.; Shimizu, S.; Smith, D. G.; Walter, J. A.; Idler D.; Khalil, W. *Can. J. Chem.* **1978**, *56*, 1898.

(16) Nakane, M.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1426.



i ii

correspond to the favored conformers. In i there are two gauche interactions around C-23, namely, C-23/C-17 and C-23/C-21, respectively, whereas in ii there is only one; C-23/C-21. This may be responsible for the upfield shift (26.90 ppm) of C-23 in i in comparison to that (33.51 ppm) of C-23 in ii. On the assumption that the C-28 methyl group in ii takes the same position as the C-23 carbon in i, one would expect a similar upfield shift mode.

In contrast to the diagnostic NMR data, which provide the most secure means of differentiation, the physical constants collected in Table III demonstrate that melting points and even rotations are practically useless, especially when dealing with very small quantities. Differences in chromatographic mobility certainly distinguish the 22-methylcholesterols (**6**, **7**) from the C-24 epimers (**4**, **5**). As far as distinction of epimers is concerned, chromatography (GC or HPLC) is effective in the 22-methyl series but useless with C-24 epimers. As expected, the mass spectra of the respective pairs (**6** vs. **7**; **4** vs. **5**) are identical. In fact, even between the two isomeric series there are insignificant mass spectral differences, which makes mass spectrometry useless for distinguishing between C-22 and C-24 methylated sterols. Consequently, we believe that even if 22-methylcholesterols (**6**, **7**) exist in nature, they probably would have escaped detection. Now that the properties of the two synthetic isomers are known, a realistic search for these biosynthetically interesting sterols in nature can be initiated.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover "Unimelt" capillary melting point apparatus and are uncorrected. Specific rotations were recorded on a Perkin-Elmer 141 polarimeter in chloroform. Column chromatography was performed on Merck column chromatography grade silica gel 60. Gas chromatography was performed on U-shaped column packed with 3% OV-17 at 260 °C. This column was mounted in a Hewlett-Packard 402 high-efficiency gas chromatograph equipped with a flame-ionization detector. HPLC was performed on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer, and a Whatman Partisil M9 10/50 ODS-2 column) with absolute methanol as the mobile phase.

^{1}H NMR spectra were recorded on a Bruker HXS-360 spectrometer with $CDCl_3$ as the solvent and Me_4Si as an internal standard and ^{13}C spectra were recorded on a Varian XL-200 spectrometer. High-resolution mass spectral data were obtained on a Varian MAT 711 spectrometer. Combined GC/MS was performed on a Varian MAT 44 instrument.

(22*E*,24*R*)-6β-Methoxy-3α,5-cyclo-5α-cholesta-22-en-24-ol (9a) and (22*E*,24*S*)-6β-Methoxy-3α,5-cyclo-5α-cholesta-22-en-24-ol (9b). (22*E*)-6β-Methoxy-3α,5-cyclo-5α-cholest-22-en-24-one (**8**; 1.3 g, 3.15 mmol) was reduced with $LiAlH_4$ (200 mg, 5.29 mmol) in THF (60 mL). After 30 min of reflux, the reaction was worked up in the usual way. The resulting alcohols **9a** and **9b** were separated on a silica gel column with the solvent system hexane–ethyl ether (20:3 v/v), yielding 0.450 g of **9a** (lower t_R) and 0.480 g of **9b** which exhibited the following properties.

9a: NMR δ 0.738 (3 H, s, 18-CH_3), 1.022 (3 H, s, 19-CH_3), 1.030 (3 H, d, J = 5.87 Hz, 21-CH_3), 5.374 (1 H, dd, J = 15.39, 6.80 Hz, 22-CH), 5.494 (1 H, dd, J = 15.40 and 8.23 Hz, 23-CH), 0.870 (3 H, d, J = 6.76 Hz, 26-CH_3), 0.915 (3 H, d, J = 6.80 Hz, 27-CH_3).

(17) Letourneaux, Y.; Khuong-Huu, Q.; Gut, M.; Lukacs, G. *J. Org. Chem.* **1975**, *40*, 1674.

Table III. Physical Constants of (22*R*)-6, (22*S*)-7, (24*R*)-4, and (24*S*)-5

sterol	mp, °C	$[\alpha]^{20}_D$ ($CHCl_3$), deg	GC rel t_R,[a] capillary, SE-54	GC rel t_R,[a] 3% OV-17	HPLC rel t_R[a] (ODS-2)
6	161-162	−40.5	1.10	1.12	0.80
7	160-161	−45.8	1.21	1.21	0.93
4	158-159	−33	1.29	1.32	1.08
5	158-159	−46	1.29	1.32	1.08

[a] Cholesterol t_R 1.00.

9b: NMR δ 0.736 (3 H, s, 18-CH_3), 1.022 (3 H, s, 19-CH_3), 1.032 (3 H, d, J = 7.29 Hz, 21-CH_3), 5.353 (1 H, dd, J = 15.38, 7.06 Hz, 22-CH), 5.448 (1 H, dd, J = 15.03, 8.20 Hz, 23-CH), 0.868 (3 H, d, J = 6.68 Hz, 26-CH_3), 0.921 (3 H, d, J = 6.74 Hz, 27-CH_3).

Both epimers had identical mass spectra: m/z (relative intensity) 414 (M^+, 7), 399 (7), 396 (5), 382 (13), 371 (7), 364 (9), 359 (23), 349 (7), 339 (64), 331 (14), 313 (7), 299 (11), 285 (18), 282 (29), 255 (80), 253 (100), 239 (16), 231 (14), 229 (22), 227 (27), 225 (16), 213 (62), 211 (27).

(24*S*)-24-Hydroxycholesterol (10a) and (24*R*)-24-Hydroxycholesterol (10b). A 40-mg sample of **9a** or **9b** dissolved in ethyl acetate (10 mL) was hydrogenated over 20 mg of 10% Pd on $BaCO_3$ followed by refluxing with *p*-toluenesulfonic acid in aqueous dioxane to give 30 mg of **10a** or **10b**.

10a: mp 180–181 °C; NMR δ 0.681 (3 H, s, 18-CH_3), 1.007 (3 H, s, 19-CH_3), 0.940 (3 H, d, J = 6.36 Hz, 21-CH_3), 0.897 (3 H, d, J = 6.77 Hz, 26-CH_3), 0.927 (3 H, d, J = 6.69 Hz 27-CH_3); ^{13}C NMR 35.849 (C-20), 18.976 (C-21), 32.133 (C-22), 30.669 (C-23), 77.306 (C-24), 33.056 (C-25), 16.643 (C-26), 18.746 (C-27).

10b: mp 185–187 °C; NMR δ 0.684 (3 H, s, 18-CH_3), 1.005 (3 H, s, 19-CH_3), 0.928 (3 H, d, J = 6.70 Hz, 21-CH_3), 0.909 (3 H, d, J = 6.93 Hz, 26-CH_3), 0.914 (3 H, d, J = 6.64 Hz, 27-CH_3); ^{13}C NMR 35.567 (C-20), 18.789 (C-21), 31.943 (C-22), 30.485 (C-23), 76.910 (C-24), 33.440 (C-25), 17.112 (C-26), 18.546 (C-27).

Both **10a** and **10b** had the same mass spectra: m/z (relative intensity) 402 (M^+,34), 384 (34), 369 (17), 351 (17), 317 (27), 314 (10), 301 (8), 300 (8), 299 (21), 291 (15), 281 (10), 273 (68), 271 (27), 255 (55), 253 (21), 245 (27), 231 (38), 229 (36), 213 (100).

Methyl (22*R*,23*E*)-6β-Methoxy-3α,5-cyclo-5α-cholest-23-en-29-oate (11a) and Methyl (22*S*,23*Z*)-6β-Methoxy-3α,5-cyclo-5α-cholest-23-en-29-oate (11b). The allylic alcohol **9a** or **9b** (100 mg, 0.24 mmol), trimethyl orthoacetate (0.31 mL, 2.4 mmol), and 2 μL of propionic acid were heated in refluxing xylenes (0.5 mL) for 3 h, when the reaction was shown to be complete by GC and TLC. After evaporation, the oily residue was purified on a silica gel column with hexane–ethyl ether (20:1), yielding 75 mg of ester **11a** or **11b**.

11a: NMR δ 0.708 (3 H, s, 18-CH_3), 1.012 (3 H, s, 19-CH_3), 0.838 (3 H, d, J = 6.70 Hz, 21-CH_3), 5.179 (1 H, dd, J = 15.50, 8.92 Hz, 23-CH), 5.385 (1 H, dd, J = 15.37, 6.72 Hz, 24-CH), 0.945 (3 H, d, J = 6.54 Hz, 26-CH_3), 0.954 (3 H, d, J = 6.66 Hz, 27-CH_3).

11b: NMR δ 0.719 (3 H, s, 18-CH_3), 1.007 (3 H, s, 19-CH_3), 5.323 (2 H, m, 23- and 24-CH), 0.930 (6 H, d, J = 7.26 Hz, 26- and 27-CH_3).

Mass spectrum for both **11a** and **11b**, m/z (relative intensity) 470 (M^+, 1.2), 455 (1.4), 438 (8), 423 (0.9), 415 (4), 407 (0.9), 315 (9), 273 (100), 255 (9), 253 (16), 241 (7), 227 (23), 215 (18), 213 (22).

Methyl (22*S*)-6β-Methoxy-3α,5-cyclo-5α-cholestan-29-oate (12a) and Methyl (22*R*)-6β-Methoxy-3α,5-cyclo-5α-cholestan-29-oate (12b). An 80-mg sample of **11a** or **11b** dissolved in ethyl acetate (15 mL) was hydrogenated over 40 mg of 10% Pd on $BaCO_3$, yielding 75 mg of **12a** or **12b**.

12a: NMR δ 0.690 (3 H, s, 18-CH_3), 1.013 (3 H, s, 19-CH_3), 0.796 (3 H, d, J = 6.59 Hz, 21-CH_3), 0.856 (3 H, d, J = 6.59 Hz, 26-CH_3), 0.874 (3 H, d, J = 6.65 Hz, 27-CH_3).

12b: NMR δ 0.732 (3 H, s, 18-CH_3), 1.018 (3 H, s, 19-CH_3), 0.768 (3 H, d, J = 6.75 Hz, 21-CH_3), 0.853 (3 H, d, J = 6.54 Hz, 26-CH_3), 0.866 (3 H, d, J = 6.52 Hz, 27-CH_3).

Both **12a** and **12b** had the same mass spectrum, m/z (relative intensity) 472 (M^+, 16), 457 (16), 440 (33), 425 (9), 417 (36), 414 (9), 319 (29), 277 (14), 273 (29), 255 (80), 245 (22), 229 (42), 213 (100).

(22S)-3β-Acetoxycholest-5-ene-28-carboxylic Acid (13a) and (22R)-3β-Acetoxycholest-5-ene-28-carboxylic Acid (13b). The i-methyl ether protecting group in **12a** and **12b** (80 mg) was removed in the usual way. The resulting products were saponified in 10 mL of 5% KOH/MeOH and then acetylated in pyridine (3 mL)–acetic anhydride (0.1 mL). The yield was 75 mg of **13a** or **13b**, which was used directly in the next step.

Phenyl (22S)-3β-Acetoxycholest-5-ene-28-carboselenoate (14a) and Phenyl (22R)-3β-Acetoxycholest-5-ene-28-carboselenoate (14b). The acetoxy acid **13a** or **13b** (45 mg, 0.09 mmol) dissolved in benzene (0.5 mL) was treated with oxalyl chloride (60 μL, 0.6 mmol) at 0 °C. After 1 h the mixture was concentrated, finally under high vacuum. To the residue dissolved in benzene (0.5 mL) was added 11 μL of pyridine and 50 μL of selenophenol. After being stirred at room temperature for 45 min, the reaction mixture was worked up in the usual manner and the crude product purified on a silica gel column with hexane–ethyl ether (20:1) to give 45 mg of **14a** or **14b**.

14a: NMR δ 0.664 (3 H, s, 18-CH_3), 1.015 (3 H, s, 19-CH_3), 0.825 (3 H, d, J = 6.73 Hz, 21-CH_3), 0.871 (3 H, d, J = 6.87 Hz, 26-CH_3), 0.890 (3 H, d, J = 6.88 Hz, 27-CH_3).

14b: NMR δ 0.703 (3 H, s, 18-CH_3), 1.024 (3 H, s, 19-CH_3), 0.794 (3 H, d, J = 6.72 Hz, 21-CH_3), 0.862 (3 H, d, J = 6.53 Hz, 26-CH_3) 0.874 (3 H, d, J = 6.61 Hz, 27-CH_3).

Both **14a** and **14b** had the same mass spectrum, m/z (relative intensity) 627 (M + 2, 0.07), 625 (M^+, 0.03), 612 (0.3), 610 (0.2), 567 (1.1), 565 (0.7), 538 (1.5), 536 (0.8), 469 (22), 451 (35), 409 (79), 391 (100), 283 (13), 255 (23), 253 (19), 213 (25), 211 (19).

(22R)-22-Methylcholesterol (6), (22S)-22-Methylcholesterol (7), (22S)-3β-Hydroxycholest-5-ene-28-carboxaldehyde (15a), and (22R)-3β-Hydroxycholest-5-ene-28-carboxaldehyde (15b). A solution of seleno ester **14a** or **14b** (70 mg, 0.11 mmol) in mesitylene (1.5 mL) was heated under reflux, after which tributyltin hydride (40 μL, 0.15 mmol) and a trace of azobis(isobutyronitrile) were added. After 15 min the mixture was concentrated and then saponified in 10 mL of 5% KOH at 64 °C for 15 min. The crude products were separated on a silica gel column with hexane/ethyl ether (20:3) and finally on HPLC with absolute methanol as the solvent. The physical constants of **6** and **7** are listed in Table III. Both **6** and **7** displayed the same high-resolution mass spectrum, m/z (relative intensity, assignment) 400.3700 (100, $C_{28}H_{48}O$, M^+), 385.3475 (11.7, $C_{27}H_{45}O$), 382.3577 (25.2, $C_{28}H_{46}$), 367.3331 (12.0, $C_{27}H_{43}$), 315.3036 (16.9, $C_{23}H_{39}$), 289.2900 (16.0, $C_{21}H_{37}$), 283.2428 (4.5, $C_{21}H_{31}$), 273.2211 (10.6, $C_{19}H_{29}O$), 255.2121 (11.8, $C_{19}H_{27}$), 231.1742 (10.0, $C_{16}H_{23}O$), 213.1657 (14.9, $C_{16}H_{21}$).

The aldehydes **15a** and **15b** exhibited the following properties.

15a: NMR δ 0.645 (3 H, s, 18-CH_3), 1.003 (3 H, s, 19-CH_3), 0.816 (3 H, d, J = 6.2 Hz, 21-CH_3), 0.867 (3 H, d, J = 6.80 Hz, 26-CH_3), 0.886 (3 H, d, J = 6.87 Hz, 27-CH_3), 9.73 (1 H, br s, 29-CH).

15b: NMR δ 0.710 (3 H, s, 18-CH_3), 1.010 (3 H, s, 19-CH_3), 0.801 (3 H, d, J = 6.73 Hz, 21-CH_3), 0.861 (3 H, d, J = 6.55 Hz, 26-CH_3), 0.868 (3 H, d, J = 6.56 Hz, 27-CH_3), 9.75 (1 H, br s, 29-CH).

Both **15a** and **15b** had the same mass spectrum, m/z (relative intensity) 428 (M^+, 18), 410 (14), 395 (11), 384 (25), 343 (14), 325 (7), 317 (9), 316 (9), 300 (14), 299 (42), 271 (42), 255 (38), 246 (22), 231 (53), 229 (40), 213 (100).

6β-Methoxy-3α,5-cyclo-22-methylene-5α-cholestane (16). Methyltriphenylphosphonium bromide (7.5 g 21 mmol) in dry benzene (150 mL) was treated with n-BuLi (21 mmol, 8.5 mL of a 2.4 N solution in hexane). After 1 h of reflux, a solution of 6β-methoxy-3α,5-cyclocholestan-22-one (1.8 g in 75 mL of benzene) was added and the reaction mixture heated for 40 h. After ether extraction and the usual workup, **16** was obtained in 90% yield after chromatography on silica gel with hexane/ether (98.5:1.5) as the eluent: NMR δ 0.742 (3 H, s, 18-CH_3), 1.027 (3 H, s, 19-CH_3), 1.035 (3 H, d, J = 6.00 Hz, 21-CH_3), 0.902 (6 H, d, J = 6.59 Hz, 26- and 27-CH_3), 4.612 and 4.694 (2 H, 2 s, 28-CH_2), 3.322 (3 H, s, 6-OCH_3); high-resolution mass spectrum, m/z (relative intensity, assignment) 412.368 49 (66.78, $C_{29}H_{48}O_1$, M^+), 397.346 58 (39.70, $C_{28}H_{45}O_1$), 380.343 82 (99.02, $C_{28}H_{44}$), 365.321 77 (18.77, $C_{27}H_{41}$), 357.317 00 (98.63, $C_{25}H_{41}O_1$), 356.309 46 (67.04, $C_{25}H_{40}O_1$), 324.283 15 (73.90, $C_{24}H_{36}$), 286.229 65 (19.56, $C_{20}H_{30}O_1$), 259.243 41 (28.06, $C_{19}H_{31}$), 255.210 85 (87.84, $C_{19}H_{27}$), 69.070 47 (100, C_5H_9).

Hydroboration of 6β-Methoxy-3α,5-cyclo-22-methylene-5α-cholestane (16). A solution of 118 mg (0.29 mmol) of **16** in 100 mL of tetrahydrofuran was cooled in an ice bath under nitrogen, and 9.5 mL of an approximately 1 M solution of diborane in tetrahydrofuran 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; 6 mL of water was added dropwise followed by 6 mL of 3 N sodium hydroxide and finally by the slow addition of 6 mL of 30% hydrogen peroxide. The mixture was stirred at room temperature for 2 h then extracted with chloroform. The combined extracts were washed with 10 mL of water followed by 5 mL of saturated sodium chloride solution and dried over magnesium sulfate. After removal of the solvent, the gummy residue was purified by preparative TLC (silica gel HF, 10% ethyl acetate in hexane as the eluent), leading to the three isomeric alcohols **17a** (4 mg), **17b** (96 mg), and **17c** (3 mg). The R_f value of **17b** was greater than that of **17a** but smaller than that of **17c**. The 360-MHz NMR data of these compounds are shown in Table I. High resolution mass spectrum of **17b**: m/z (relative intensity, assignment) 430.378 30 (84.99, $C_{29}H_{50}O_2$, M^+), 415.356 09 (39.81, $C_{28}H_{47}O_2$), 398.354 93 (100, $C_{28}H_{46}O_1$), 375.324 95 (73.39, $C_{25}H_{43}O_2$), 372.338 85 (27.63 $C_{26}H_{44}O_1$), 277.253 87 (10.99, $C_{19}H_{33}O_1$), 255.211 15 (23.10, $C_{19}H_{27}$). The mass spectrum of **17a** was indistinguishable from that of **17b**.

Acetylation (acetic anhydride, pyridine, room temperature, 12 h) gave the respective acetates whose 360-MHz NMR properties are as follows.

Acetate of **17b**: ^{1}H NMR δ 0.721 (3 H, s, 18-CH_3), 1.022 (3 H, s, 19-CH_3), 0.834 (3 H, d, J = 6.78 Hz, 21-CH_3) 0.880 (3 H, d, J = 6.64 Hz, 26-CH_3), 0.888 (3 H, d, J = 6.58 Hz 27-CH_3), 3.811 (1 H, dd, J = 10.94, 8.16 Hz), 4.129 (1 H, dd J = 10.95, 4.32 Hz, 28-CH_2).

Acetate of **17c**: ^{1}H NMR δ 0.721 (3 H, s, 18-CH_3), 1.017 (3 H, s, 19-CH_3), 0.754 (3 H, d, J = 7.03 Hz, 21-CH_3), 0.886 (3 H, d, J = 6.61 Hz, 26-CH_3), 0.890 (3 H, d, J = 6.85 Hz, 27-CH_3), 3.869 (1 H, dd, J = 10.86, 8.62 Hz), 4.127 (1 H, dd, J = 10.91, 4.19 Hz, 28-CH_2).

(22S)-22-Methylcholesterol (7). A solution of 83 mg (0.2 m mol) of the 22S alcohol **17b** in 12 mL of dry pyridine was cooled in an ice bath, and 2.4 mL of methanesulfonyl chloride was added dropwise with stirring. After being stirred overnight, the mixture was poured into 100 mL of ice–water and extracted with three 50-mL portions of chloroform. After the usual workup the crude mesylate (**18b**) was taken up in 90 mL of dry tetrahydrofuran. Excess lithium aluminum hydride was added, and the mixture was heated to reflux with stirring overnight. Excess lithium aluminum hydride was destroyed with water, the product extracted with ether, and the residue purified by preparative TLC (silica gel HF, 8% ethyl acetate in hexane as the eluent) to give 11.5 mg (15%) of the i-methyl ether of (22S)-22-methylcholesterol (**19b**).

A 10-mg sample of **19b** in 1.5 mL of 10% aqueous dioxane containing p-toluenesulfonic acid (0.5 mg) was heated under reflux for 1 h and diluted with water, and the solid (22S)-22-methylcholesterol (**7**) was recrystallized from methanol to give pure **7** (5.8 mg, 60%; mp 160–161 °C) identical with material prepared by the Claisen route.

(22R)-22-Methylcholesterol (6). A 2.6-mg sample of the 22R alcohol **17a** was treated exactly as described above to provide (22R)-22-methylcholesterol (mp 161–162 °C) which proved to be identical with a specimen prepared by Claisen rearrangement.

(20S)-22ζ-Methylcholesterol (20). A 3-mg sample of **17c** was converted to the mesylate and reduced with lithium aluminum hydride, and the i-methyl ether protecting group was removed to afford **20**: NMR δ 0.742 (3 H, s, 18-CH_3), 1.008 (3 H, s, 19-CH_3), 0.676 (3 H, d, J = 6.70 Hz, 21- or 28-CH_3), 0.873 (3 H, d, J = 6.97 Hz, 26-CH_3), 0.875 (3 H, d, J = 6.37 Hz, 27-CH_3), 0.730 (3 H, d, J = 6.77 Hz, 28- or 21-CH_3); high-resolution mass spectrum; m/z (relative intensity, assignment) 400.370 58 (100, $C_{28}H_{48}O_1$, M^+), 385.345 69 (13.36, $C_{27}H_{45}O_1$), 382.358 74 (27.93, $C_{28}H_{46}$), 367.332 19 (12.44, $C_{27}H_{43}$), 315.305 76 (20.46, $C_{23}H_{39}$), 289.289 64 (15.76, $C_{21}H_{37}$), 273.220 83 (10.97, $C_{19}H_{29}O_1$), 225.209 16 (11.01, $C_{19}H_{27}$), 213.163 96 (12.63, $C_{16}H_{21}$).

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Registry No. **6**, 80082-93-5; **7**, 80082-94-6; **8**, 55064-55-6; **9a**, 80082-95-7; **9b**, 80082-96-8; **10a**, 474-73-7; **10b**, 27460-26-0; **11a**, 80082-97-9; **11b**, 80082-98-0; **12a**, 80082-99-1; **12b**, 80083-00-7; **13a**, 80105-70-0; **13b**, 80083-01-8; **14a**, 80083-02-9; **14b**, 80083-03-0; **15a**, 80083-04-1; **15b**, 80083-05-2; **16**, 79396-52-4; **17a**, 80083-06-3; **17b**, 80083-07-4; **17b** acetate, 80083-08-5; **17c**, 80125-09-3; **17c** acetate, 80125-10-6; **18a**, 80083-09-6; **18b**, 80083-10-9; **18c**, 80125-11-7; **19a**, 80083-11-0; **19b**, 80083-12-1; **19c**, 80125-12-8; **20**, 80125-13-9; 6β-methoxy-3α,5-cyclocholestan-22-one, 80083-13-2.

Synthesis of Sterols with Cyclopropane-Containing Side Chains. Spectroscopic Properties and Absolute Configurations

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The synthesis of seven Δ^5 sterols with cyclopropane-containing side chains (**1–3** and **5–8**) by means of dichlorocarbene addition to the appropriate olefinic precursor is described. Separation of the diastereoisomeric mixtures of the primary dichlorinated adducts was accomplished by reverse-phase high-performance liquid chromatography. The effect of stereochemistry upon the NMR and mass spectroscopic properties of the diastereoisomerically pure sterols are reported, and their absolute configurations were determined either by X-ray crystal structure analysis of related precursors and/or by chemical and spectroscopic correlations.

Sterols with cyclopropane-containing side chains may be key intermediates in bioalkylation processes.[1,2] Indeed, speculations about their possible biosynthetic role had been advanced by Lederer[3] prior to the isolation of any such sterols in nature. Furthermore, they are useful as chemical tracers[4] in following complicated food chains in the marine environment. Our own studies in the marine sterol field,[1,2] especially those dealing with the occurrence of new cyclopropanated sterols,[5–8] prompted us to examine the synthesis of various suitable model compounds.[5,9–11] In order to examine the effect of stereochemical changes around the cyclopropane ring upon chemically or biologically induced isomerization or ring-opening reactions, we were especially interested in different cyclopropanated sterols with defined absolute configurations. In the present paper, we report the details of the synthesis and isolation of seven steroidal cyclopropanes, **1–3** and **5–8** (Chart I), which are of actual or potential relevance and of the hitherto unknown (23*Z*)-cholesta-5,23-dien-3β-ol (**19**).[12] These syntheses are also useful for the preparation of isotopically labeled analogues which may be required for biosynthetic studies. Additionally, we present their spectroscopic properties, notably the NMR data, which allow us to draw conclusions about the absolute configurations of **1** and **2** in comparison with **5** and **6**. Conformational assignments for the sterols **5–8** are based on an X-ray study of related trichloro steroids, which will be published elsewhere.[13]

Results and Discussion

The synthetically more attractive stereospecific routes[5,10,14,15] to diastereoisomerically pure, substituted, three-membered rings in the sterol side chain either failed

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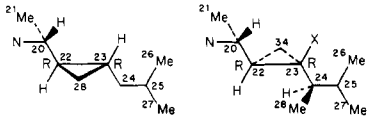
(5) Blanc, P.-A.; Djerassi, C. *J. Am. Chem. Soc.* **1980**, *102*, 7113–7114. In this paper, we correctly showed that the absolute configuration of naturally occurring 23,23-methylenecholesterol (i) is opposite to that



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(22*R*,23*R*,24*R*) of gorgosterol (ii, X = CH_3) and therefore identified the natural material as the 22*S*,23*S* isomer. In fact, this is a misapplication of the Cahn–Ingold–Prelog rules (*Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–415): even though of opposite absolute configuration, the natural 22,23-methylenecholesterol (i) should really be referred to as 22*R*,23*R*. This is so because the 22*R* indication in the related sterols gorgosterol (ii, X = CH_3) and demethylgorgosterol (ii, X = H) is based on a higher priority of C-23 over C-20, which is for demethylgorgosterol due to methyl substitution on C-24; 23*R* then results from the priority of C-22 over C-24. However, *lack of the C-28 methyl group in 22,23-methylenecholesterol (i) leads to an inversion of the sequences around C-22 and C-23.* Regarding the 22-carbon atom, C-20 now assumes priority over C-23, while the sequence around C-23 is H < C-24 < C-28 < C-22. Apparently the *R,S* convention is subject to some uncertainty in the case of sterols with cyclopropane-containing side chains. The *R,S* notation for the 23-carbon atom in 23,24-methylenecholesterol, for example, depends on what priority is assigned to C-24 vs. C-20. In this particular case, we interpret the Cahn–Ingold–Prelog "always-outward" rule in terms of C-20 > C-24.

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