# Structural and Stereochemical Aspects of Base-Catalyzed Double Bond Isomerization of Sterols with Unsaturated Side Chains

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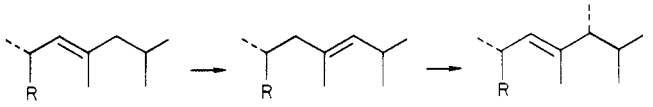
A careful analysis of the *N*-lithioethylenediamine-catalyzed isomerization of 22-, 23-, and 24-methylenecholesterol demonstrated the existence of subtle factors which are responsible for the kinetically and thermodynamically preferred products. In general, the results can be related to the anticipated energy preferences of the intermediate carbanions. Through the use of $^1H$ and $^{13}C$ NMR spectroscopy the configuration of the various double bond isomers could be established. Since all major products are readily separated by HPLC, such base-catalyzed isomerization offers a convenient synthetic route to a variety of stereochemically pure sterol olefins, which either have been found in the marine environment or may be detected in the future.

The isolation[2] of 22(*R*),23(*R*)-methylenecholesterol (**3**) from various marine animals constituted the first evidence for the direct bioalkylation of 22-dehydrocholesterol (**1**). Our recent isolation[3] of 22-methylenecholesterol (**6**) represented further indirect support for such bioalkylation. A possible pathway involving *S*-adenosylmethionine (SAM) is outlined in Scheme I.

Moreover, the occurrence of 23-methyl-22-dehydrocholesterol (**7**)[4,5] and its 4-methyl analogue (**8**)[6] in marine organisms is also compatible with direct bioalkylation of the $\Delta^{22}$ double bond. This raises two questions.[7] If 22-methylenecholesterol (**6**) and the 23-methyl-22-dehydro sterols **7** and **8** arise directly from 22-dehydrocholesterol (**1**), is it possible that such SAM biomethylation (cf. Scheme I) produces also 22-methyl-22-dehydrocholesterol (**9**)? Since 23-methyl-22-dehydro sterols (**7**, **8**) exist in nature, is it possible that the dinosterol (**11**) type side chain is formed by in vivo double bond migration of **7** or **8** to the hitherto unknown 23-methyl-23-dehydro sterols (**10**),



7, R = A
8, R = C

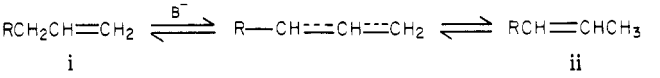
10

11, R = C

followed by a second biomethylation rather than by the generally assumed reverse sequence (biomethylation at C-24 followed by methylation at C-23?

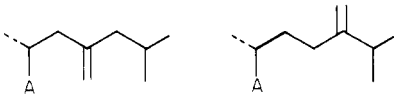
Therefore, a search for such biogenetic "missing links" is of obvious relevance, and in order to facilitate the detection of these hitherto unknown sterols, we undertook their synthesis by base-catalyzed isomerization of readily available unsaturated precursors.

The base-catalyzed isomerization of olefins is closely related to the chemistry of carbanions, with a thermodynamically less stable olefin being transformed into a more stable one. Thus a terminal olefin (i) affords the internal isomer (ii):



i ii

Various base catalysts and solvents have been examined for this type of isomerization.[8-16] Alkali metals and their alcoholates or amides are particularly effective catalysts; dimethyl sulfoxide ($Me_2SO$), dimethylformamide (DMF), hexamethylphosphoric triamide (HMPT), glycol ethers, amines, and ammonia can be employed as solvents. An early report[17] indicated that *N*-lithioethylenediamine in ethylenediamine is much more active than sodioethylenediamine in shifting a terminal double bond into an internal position of an olefin. This reagent has also been studied for the same purpose in the field of terpenoids and sterols.[18-21] Therefore, we selected *N*-lithioethylenediamine as the base catalyst for the isomerization of 22-methylenecholesterol (**6**),[3] 23-methylenecholesterol (**12**),[22]



12 13

and 24-methylenecholesterol (**13**)[23] and subjected the reaction mixtures to careful analysis by HPLC (see Tables I, II, and IV). As shown in the sequel, interesting differences could be detected among those closely related isomers.

Exposure of 22-methylenecholesterol (**6**) to *N*-lithioethylenediamine for 10 min led largely to recovered starting material. However, after 2 h (see Table I) there

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(2) Blanc, P.-A.; Djerassi, C. *J. Am. Chem. Soc.* **1980**, *102*, 7113-7114; **1981**, *103*, 7036.
(3) Zielinski, J.; Li, H.-t.; Milkova, T. S.; Popov, S.; Marekov, N. L.; Djerassi, C. *Tetrahedron Lett.* **1981**, 2345-2348.
(4) Kokke, W. C. M. C.; Withers, N. W.; Massey, I. J.; Fenical, W. H.; Djerassi, C. *Tetrahedron Lett.* **1979**, 3601-3604.
(5) Kobayashi, M.; Tomioka, A.; Hayashi, T.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1979**, *27*, 1951-1953.
(6) Alam, M.; Schram, K. H.; Ray, S. M. *Tetrahedron Lett.* **1978**, 3517-3518.
(7) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873-890.
(8) Hubert, A. J.; Reimlinger, H. *Synthesis* **1969**, 97-112.
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(10) Schriesheim, A.; Hofman, J. E.; Rowe, C. A., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 3731-3732.
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(12) Schriesheim, A.; Rowe, C. A., Jr. *J. Am. Chem. Soc.* **1962**, *84*, 3160-3164.
(13) Schriesheim, A.; Rowe, C. A., Jr.; Naslund, L. *J. Am. Chem. Soc.* **1963**, *85*, 2111-2115.
(14) Asinger, F.; Fell, B.; Krings, P. *Chem. Ber.* **1966**, *99*, 1737-1741.
(15) Reggel, L.; Friedel, R. A.; Wender, I. *J. Org. Chem.* **1957**, *22*, 891-894.
(16) David, L.; Kergomard, A.; Vincent, S. *C. R. Hebd. Seances Acad. Sci. Ser. C* **1968**, *266*, 338-340.
(17) Reggel, L.; Friedman, S.; Wender, I. *J. Org. Chem.* **1958**, *23*, 1136-1139.
(18) Narula, A. S.; Dev, S. *Tetrahedron* **1971**, *27*, 1119-1127.
(19) Singh, C.; Singh, J.; Dev, S. *Tetrahedron* **1977**, *33*, 1759-1763.
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(21) Ayanoglu, E.; Chan, A.; Djerassi, C. *Tetrahedron* **1979**, *35*, 1591-1594.
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(23) Idler, D. R.; Fagerlund, U. H. M. *J. Am. Chem. Soc.* **1957**, *79*, 1988-1991.

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Scheme I

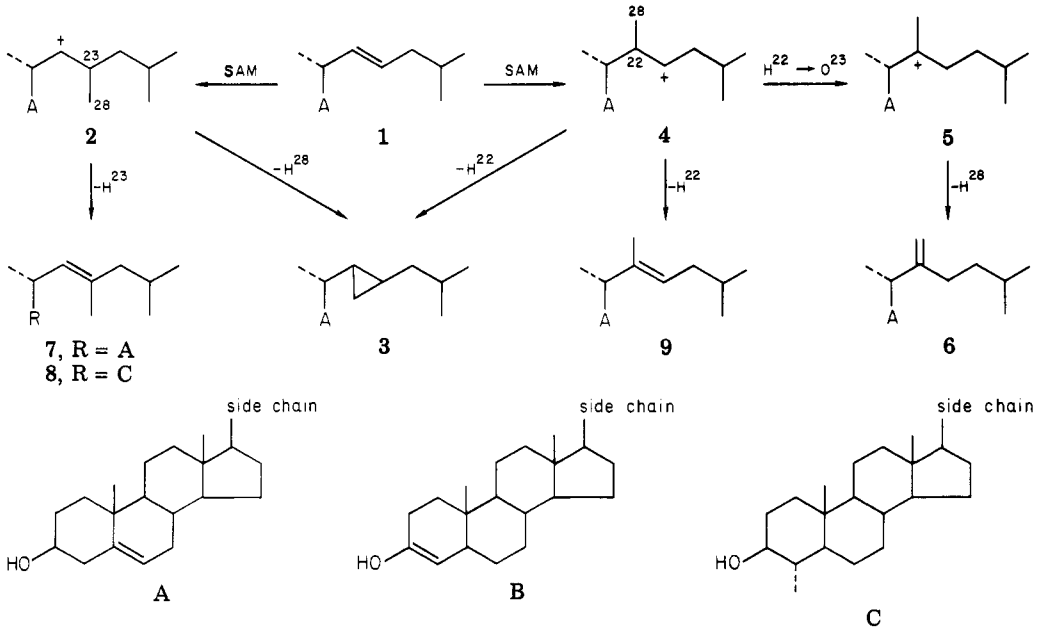



Table I. Composition from the Isomerization of 22-Methylenecholesterol (6)

| reaction period, h | component 6 | 9 | 16 | 17 | 18 |
|---|---|---|---|---|---|
| 2 | 6 | 78 | 6 | 8 | |
| 24 | 7 | 55 | 15 | 13 | 6 |
| 40[a] | | 64 | 15 | 9 | |

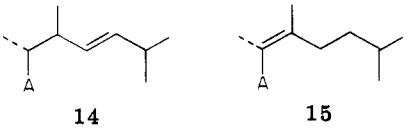
[a] Using 9 as the starting material.

Table II. Percent Composition from the Isomerization of 23-Methylenecholesterol (12)

| reaction period | component 7 | 26 | 25 | 23 | 24 | 12 |
|---|---|---|---|---|---|---|
| 10 min | 29 | 2 | | 60 | | 9 |
| 0.5 h | 55 | 9 | 2 | 23 | | 11 |
| 2 h | 63 | 11 | 5 | 10 | | 9 |
| 0.5 h[a] | 51 | 7 | | 31 | | 9 |
| 0.5 h[b] | 80 | 4 | | 11 | | 5 |

[a] Using 23 as the starting material. [b] Using 7 as the starting material. [c] For 25 and 26.

was obtained 78% of the desired (22*E*)-22-methyl-22-dehydrocholesterol (**9**) and 6% of its 22*Z* isomer **16**, together with 6% of starting material and 8% of the $\Delta^4$ isomer **17**. Extending the reaction time to 24 h increased slightly the proportion of **16** and **17** at the expense of the principal rearrangement product **9**. However, neither the disubstituted olefin **14** nor the tetrasubstituted olefin **15**



14 15

was formed, even when the 22*E* isomer **9** was heated with *N*-lithioethylenediamine for 40 h. The reason the disubstituted olefin **14** is not formed must be related to carbanion stability, viz., primary > secondary > tertiary.[10,13]

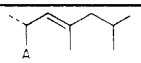
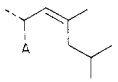
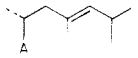
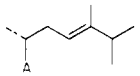
The stereochemistry of the $\Delta^{22}$ double bond in **9** and **16** could be established by comparing their $^1$H NMR spectral data (Table V) with that of *cis*- and *trans*-4-methyl-2-pentene.[24]

The chemical shift for *cis*- (iv) and *trans*-4-methyl-2-pentene (iii) indicated that the isopropyl methine proton

iii iv

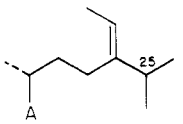
(24) Frost, D. J.; Ward, J. P. *Tetrahedron Lett.* **1968**, 3779–3782. Erdman, T. R.; Scheuer, P. J. *Lloydia* **1975**, *38*, 359–360.

Table III. Selected $^{13}C$ Chemical Shifts of Some Side Chain Unsaturated Sterols

| compd | chemical shift, ppm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | C-20 | C-21 | C-22 | C-23 | C-24 | C-25 | C-26 | C-27 | C-28 |
|  7 | 34.97 | 20.73 | 133.26 | 130.46 | 49.74[a] | 26.09 | 22.32 | 22.58 | 16.27[b] |
|  26 | 34.30 | 20.79 | 133.34 | 130.65 | 41.34 | 26.23 | 22.24 | 22.84 | 23.47 |
|  23 | 34.06 | 17.91 | 46.92 | 131.43 | 134.43 | 27.16 | 23.12 | 23.33 | 15.89 |
|  29 | 36.86 | 18.71 | 34.21 | 120.74 | 124.87 | 36.95 | 21.55 | 21.55 | 13.52 |

[a] Lit.[5] 49.6 ppm. [b] Lit.[5] 16.2 ppm.

in the trans isomer is at higher field ($\delta$ 2.3) than that in the cis isomer ($\delta$ 2.6). The same observation has been made in comparing the chemical shifts of the C-25 proton of fucosterol (**19**, $\delta$ 2.2) with that ($\delta$ 2.8) of isofucosterol (**20**).[24]



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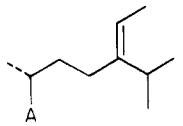

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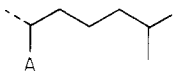
Since the chemical shift of the C-20 proton in **9** occurs (Table V) at 2.065 ppm and in **16** at 2.660 ppm, the isopropyl methine of **9** should be cis oriented to the C-22 H, thus leading to the *E* configuration for the $\Delta^{22}$ double bond in **9** and the *Z* configuration in **16**. The structures of the isomers **17** and **18** were elucidated by $^1H$ NMR spectroscopy. The signals of the appropriate protons in the side chain of **17** and **18** are identical with those of **9** and **16**, respectively; therefore, their side chains are identical. In addition, **17** and **18** display (see Table V) the NMR characteristics of an allylic alcohol, indicating that the $\Delta^5$ double bond had also migrated to the $\Delta^4$ position. In order to prove this supposition, we heated cholesterol (**21**) with



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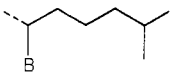

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*N*-lithioethylenediamine for 40 h, whereupon a 19% yield of cholest-4-en-3$\beta$-ol (**22**)[25] was isolated. Clearly the lithium reagent also has the ability to transform a $\Delta^5$ double bond of $\Delta^5$-3-hydroxy sterols into the $\Delta^4$ isomer, although the reaction is much slower than that of the terminal double bond in the side chain.

The proportion of different isomers resulting from the base-catalyzed isomerization of 23-methylenecholesterol (**12**) under various reaction periods is outlined in Table II. Even after 10 min, when most of the 22-methylenecholesterol (**6**) was recovered (see Table I), the 23-methylene isomer **12** was already transformed to the extent of 90% to the double bond isomers **7**[4,5] and **23**. (It is pertinent to note that when *N*-lithioethylenediamine was replaced by the bulkier lithium diisopropylamide, only starting material was recovered after 6 h.) The relative ratio of the $\Delta^{22}$ (**7**) and $\Delta^{23}$ (**23**) isomers changes dramatically upon increasing the reaction time, thus showing that **23** is the kinetically favored and that **7** is the thermodynamically favored isomer. The stability of these isomers also could be established (cf. Table II) by comparing the reaction mixture composition after treating the pure $\Delta^{22}$ (**7**) and $\Delta^{23}$ (**23**) olefins separately with the lithium catalyst system. The thermodynamic preference of the $\Delta^{22}$-23-methyl isomer **7** over its $\Delta^{23}$ counterpart **23** may be the explanation why only the former has so far been encountered in nature.[4-6]

The *location* of the double bond in these isomers could be determined by $^1H$ NMR spectroscopy (cf. Table V) and by their high-resolution mass spectra (see Table VII for *m/z* 300 base peak associated with a McLafferty-type rearrangement of the $\Delta^{23}$ double bond). The *stereochemistry* of the double bond in each isomer was established by $^{13}C$ NMR spectroscopy (Table III) in the following manner.

The $^{13}C$ chemical shifts of a methyl or methylene group in a trisubstituted olefin have already been discussed in the literature.[26] If the methyl or methylene group is located cis to the olefinic hydrogen atom, its $^{13}C$ NMR signal shows a downfield shift, whereas a trans relationship results in an upfield shift. With methyl groups the chemical shift difference amounts to ca. 8 ppm, while between two methylene groups it is nearly 8.6 ppm. In our sterol isomers the chemical shift difference of the C-28 methyl groups is 7.2 ppm (16.27 ppm in **7** vs. 23.47 ppm in **26**) and 8.4 ppm for the C-24 methylene groups (49.74 ppm in **7** vs. 41.34 ppm in **26**). Therefore, we can assume with confidence that the C-28 methyl group is located trans to the C-22 hydrogen atom in **7** and cis in **26**. Similarly, the $^{13}C$ NMR signal of the C-28 methyl group in the $\Delta^{23}$ isomer **23** occurs at 15.98 ppm, whereupon it follows that the $\Delta^{23}$ double bond in **23** must have the *E* configuration.

Since 23-methyl-22-dehydrocholesterol (**7**) can be transformed partially into its 23-dehydro isomer **23** by base isomerization, though the reverse reaction (see last two entries in Table II) occurred easily, it was of interest to see what would happen to the more highly substituted dinosterol (**11**). After a 40-h reaction period 74% of recovered dinosterol (**11**) was encountered in addition to 18% of the 24$\beta$-methyl isomer **28**.[27] None of the presumed intermediate tetrasubstituted isomer **27** could be detected.

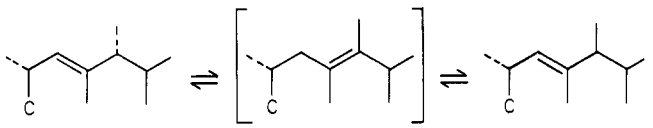
The isomerization of the well-known 24-methylenecholesterol (**13**) has been studied in the past by several groups.[28-30] In each instance, iodine–benzene was em-

(25) Cacchi, S.; Giannoli, B.; Misiti, D. *Synthesis* **1974**, 728–730.

(26) Dorman, D. E.; Jautelat, M.; Roberts, J. D. *J. Org. Chem.* **1971**, *36*, 2757–2766.

(27) Shu, A. Y. L.; Djerassi, C. *Tetrahedron Lett.* **1981**, 4627–4630.

(28) Sakai, K. *Chem. Pharm. Bull.* **1963**, *11*, 752–754.



11 27 28

ployed as the catalytic system, and two isomers (**29** and **31**)[28,31] were reported to be formed. When the *N*-lithioethylenediamine catalyst is applied, the double bond of the 24-methylene group is easily isomerized. Even after 30 min, none of the starting material could be detected. As shown in Table IV, the $\Delta^{23}$ isomer **29** predominates initially, but when the reaction period is extended to 2 h, the tetrasubstituted $\Delta^{24}$ olefin **31** becomes dominant. Small amounts of the 23*Z* isomer **30**, codisterol (**32**),[32] and epicodisterol (**33**)[33] could also be isolated in each instance.

The configuration of the $\Delta^{23}$ double bond in **29** and **30** could be assigned by $^{13}C$ NMR spectroscopy on the basis of the chemical shift of the C-28 methyl group (cf. Table III). Furthermore, in Table V, the $^{1}H$ NMR data also show that in all of these trisubstituted steroidal olefins, the chemical shifts of the C-28 methyl group situated cis to the olefinic proton are found downfield, whereas a trans relationship results in an upfield shift. This is in full concordance with the results from the $^{13}C$ NMR studies (Table III).

In Table VI we list the relative retention times for HPLC and GC since these should prove to be particularly useful when searching for the possible presence of some of these novel sterols among marine natural sources. A comparison of the polarity of *E* and *Z* isomeric pairs on HPLC shows that the sterols with a *Z*-configured double bond are more polar than their *E* counterparts.

Finally, we list in Table VII the relative intensities of selected mass spectral fragments to aid in the eventual detection of some of these sterols by GC/MS techniques. The main conclusions can be summarized as follows.

(1) All of the three methylene cholesterols (**6**, **12**, **13**) display a base peak (*m/z* 342 vs. 300 vs. 314) due to McLafferty rearrangements. This is not true of terminal $\Delta^{25}$ double bonds [e.g., codisterol (**32**) and epicodisterol (**33**)] which show practically no McLafferty rearrangement ion at *m/z* 328 but rather possess the parent ion as the base peak.

(2) The base peak of cholesta-5,22-dien-3$\beta$-ol (**1**) is at *m/z* 55 as reported previously.[34] When a methyl group is substituted at C-22 or C-23, irrespective of the *E* or *Z* double bond configuration, all of these four sterols (**9**, **16**, **7**, **26**) have the same base peak at *m/z* 69, thus indicating that these hydrocarbon fragments may be associated with the side chain. The intensities of the molecular and [M − side chain + H] ions (*M/z* 272) of the 22-methyl compounds (**9**, **16**) are higher than those of the 23-methyl compounds (**7**, **26**), but the intensities of *m/z* 300 (vinylic cleavage) in the 22-methyl isomers are slightly lower than those in the 23-methyl series (**7**, **26**).

(3) The molecular ion peak of cholesta-5,23(*Z*)-dien-3$\beta$-ol (**34**) is the base peak, but the peak (*m/z* 271) corresponding to [M − side chain + 2H] is of almost equal intensity (97%).[35] When a methyl group is introduced into the C-23 or C-24 positions, irrespective of the double bond configuration, the intensities of the parent ions of all these four sterols (**23**, **24**, **29**, **30**) are greatly reduced. The base peaks of the two 23-methyl compounds (**23**, **24**) occur at *m/z* 300 and are assumed to arise by McLafferty rearrangement ($\beta$-cleavage). The peaks at *m/z* 398 (parent ion) and *m/z* 271 are of very low intensities. The base peaks of the two 24-methyl sterols (**29**, **30**) occur at *m/z* 271, caused by side chain loss plus two hydrogens.

(4) The mass spectrum of 24-methyl-$\Delta^{24}$-cholesterol (**31**) is in agreement with earlier reports[36,37] and features an intense McLafferty rearrangement ion at *m/z* 314.

In summary, the *N*-lithioethylenediamine-catalyzed isomerization of side-chain methylene-substituted sterols is of considerable preparative utility for a variety of unsaturated side-chain-methylated sterols. Their availability should facilitate the search for such biogenetic missing links among marine organisms, and the methods described in this paper should also lend themselves to convenient isotopic labeling for bioincorporation studies.

## 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. Gas chromatography was performed on an 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. Capillary gas chromatography was performed on a Carlo Erba Series 4160 Fractovap chromatograph equipped with a Model 400 LT programmer and a 15 m × 0.32 mm fused silica column coated with SE-54 at 260 °C. 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 equipped with a Nicolet TT 1010-A computer with $CDCl_3$ as the solvent and $Me_4Si$ as an internal standard. $^{13}C$ spectra were recorded on a Varian XL-200 spectrometer. Chemical shifts are given in parts per million and *J* values in hertz. High-resolution mass spectral data were obtained on a Varian MAT 711 spectrometer.

**General Procedure for Isomerization of Sterols.** A solution of *N*-lithioethylenediamine in ethylenediamine prepared from 0.2 g of lithium (containing 0.02% of Na) and 8 mL of anhydrous ethylenediamine under nitrogen at 90 °C was added to 30–50 mg of sterol. The mixture was stirred at 115 °C under nitrogen for 10 min to 40 h (see Tables I, II and IV), poured into ice-water, and extracted with ether (3 × 15 mL). The combined extracts were washed with 10% HCl, saturated aqueous sodium bicarbonate, and water and finally dried over anhydrous sodium sulfate. Removal of the ether under reduced pressure gave a crude solid which was subjected to separation by reverse-phase HPLC.

**Isomerization of 22-Methylenecholesterol (6).** 22-Methylcholesta-5,22(*E*)-dien-3$\beta$-ol (**9**) and 22-methylcholesta-4,22(*Z*)-dien-3$\beta$-ol (**18**) were obtained in pure form by separation on an ODS-2 column. 22-Methylcholesta-5,22(*Z*)-dien-3$\beta$-ol (**16**) and 22-methylcholesta-4,22(*E*)-3$\beta$-ol (**17**) had the same retention time on an ODS-2 column; therefore, they had to be separated again on an ODS-3 column with 95% methanol as the solvent.

**22-Methylcholesta-5,22(*E*)-dien-3$\beta$-ol (9):**[38] mp 148–150 °C (MeOH); $[\alpha]^{20}_{D}$ −64°; high-resolution mass spectrum; *m/z* (relative intensity, assignment) 398.356 54 (67, $C_{28}H_{46}O$, $M^+$), 383.332 24 (2, $C_{27}H_{43}O$), 380.346 15 (12, $C_{28}H_{44}$), 342.291 97 (1, $C_{24}H_{38}O$),

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Table IV. Percent Isomerization of 24-Methylenecholesterol (13)

| reaction period, h | component 29 | 30 | 31 | 32 | 33 |
|---|---|---|---|---|---|
| 0.5 | 52 | 3 | 38 | 2 | 2 |
| 2 | 38 | 7 | 43 | 4 | 4 |

Table V. 360-MHz $^1$H NMR Data ($CDCl_3$)[a] of 22-, 23-, and 24-Methyl Sterols

| sterol | chemical shift 18-Me | 19-Me | 21-Me | 26-Me | 27-Me | 28-Me | other signals |
|---|---|---|---|---|---|---|---|
| 6 | 0.705 | 1.016 | 1.040 ($J$ = 6.75) | 0.902 ($J$ = 6.57) | 0.902 ($J$ = 6.57) | | C28 H, 4.615, 4.696 |
| 9 | 0.700 | 1.014 | 1.016 ($J$ = 6.50) | 0.866 ($J$ = 6.42) | 0.876 ($J$ = 6.46) | 1.470 | C20 H, 2.065; C23 H, 5.120 |
| 16 | 0.742 | 1.023 | 0.982 ($J$ = 6.71) | 0.870 ($J$ = 6.73) | 0.891 ($J$ = 6.89) | 1.579 | C20 H, 2.660; C23 H, 4.995 |
| 17 | 0.698 | 1.053 | 1.001 ($J$ = 6.78) | 0.862 ($J$ = 6.64) | 0.871 ($J$ = 6.63) | 1.461 | C23 H, 5.113; C3 H, 4.15; C4 H, 5.274 |
| 18 | 0.739 | 1.061 | 0.969 ($J$ = 6.72) | 0.870 ($J$ = 6.65) | 0.891 ($J$ = 6.74) | 1.573 | C20 H, 2.599; C23 H, 4.973; C3 H, 4.15; C4 H, 5.276 |
| 12 | 0.709 | 1.012 | 0.904 ($J$ = 6.04) | 0.833 ($J$ = 5.99) | 0.857 ($J$ = 5.71) | | C28 H, 4.691, 4.709 |
| 7 | 0.717 | 1.013 | 0.942 ($J$ = 6.58) | 0.812 ($J$ = 6.19) | 0.836 ($J$ = 6.28) | 1.558 | C22 H, 4.876 |
| 26 | 0.707 | 1.014 | 0.927 ($J$ = 6.52) | 0.844 ($J$ = 6.41) | 0.894 ($J$ = 6.35) | 1.610 | C22 H, 4.945 |
| 23 | 0.701 | 1.010 | 0.803 ($J$ = 6.04) | 0.926 ($J$ = 6.70) | 0.926 ($J$ = 6.70) | 1.546 | C24 H, 4.904 |
| 24 | 0.703 | 1.013 | 0.827 ($J$ = 6.51) | 0.891 ($J$ = 6.59) | 0.909 ($J$ = 6.56) | 1.602 | C24 H, 4.993 |
| 25 | 0.714 | 1.052 | 0.928 ($J$ = 6.58) | 0.808 ($J$ = 6.08) | 0.833 ($J$ = 6.25) | 1.552 | C22 H, 4.866; C3 H, 4.15; C4 H, 5.2 |
| 13 | 0.684 | 1.011 | 0.950 ($J$ = 6.18) | 1.023 ($J$ = 6.50) | 1.027 ($J$ = 6.50) | | C28 H, 4.657, 4.713 |
| 29 | 0.686 | 1.008 | 0.888 ($J$ = 6.55) | 0.985 ($J$ = 6.84) | 0.985 ($J$ = 6.84) | 1.543 | C23 H, 5.138 |
| 30 | 0.682 | 1.008 | 0.903 ($J$ = 6.49) | 0.947 ($J$ = 6.84) | 0.947 ($J$ = 6.84) | 1.602 | C23 H, 5.027; C25 H, 2.825 |
| 31 | 0.681 | 1.009 | 0.960 ($J$ = 6.49) | 1.630 | 1.630[b] | 1.617[b] | |
| 32 | 0.671 | 1.006 | 0.910 ($J$ = 6.52) | 1.636 | | 0.992 ($J$ = 7.01) | C27 H, 4.658 |
| 33 | 0.673 | 1.007 | 0.915 ($J$ = 6.56) | 1.650 | | 0.985 ($J$ = 6.91) | C27 H, 4.655 |

[a] Shifts are given in parts per million and $J$ values in hertz. [b] Assignment could be reversed.

Table VI. Chromatographic Data of 22-, 23-, and 24-Methyl Sterols

| sterol | rel $t_R$[a] HPLC (ODS-2) | GC (3% OV-17) | capilliary GC (SE-54) |
|---|---|---|---|
| 6 | 0.66 | 1.13 | 1.09 |
| 9 | 0.71 | 1.20 | 1.11 |
| 16 | 0.61 | 0.94 | 1.00 |
| 17 | 0.61 | b | b |
| 18 | 0.53 | b | b |
| 12 | 0.82 | 1.19 | 1.12 |
| 7 | 0.89 | 1.07 | 1.08 |
| 26 | 0.75 | 0.99 | 1.00 |
| 23 | 0.98 | 1.18 | 1.11 |
| 24 | 0.84 | 1.13 | 1.08 |
| 25 | 0.75 | b | b |
| 13 | 0.80 | 1.34 | 1.30 |
| 29 | 0.84 | 1.35 | 1.32 |
| 30 | 0.80 | 1.42 | 1.40 |
| 31 | 0.90 | 1.63 | 1.52 |
| 32 | 0.77 | 1.26 | |
| 33 | 0.80 | 1.25 | |

[a] Cholesterol $t_R$ = 1.00. [b] Decomposes at 260 °C under GC conditions.

Table VII. Relative Intensities (in Percent) of Selected Mass Spectral Fragments

| sterol | $m/z$ 398 (M) | 314 | 300 | 299 | 272 | 271 | other $m/z$ (rel intens) |
|---|---|---|---|---|---|---|---|
| 6 | 27 | | 3 | 3 | 74 | 10 | 342 (100) |
| 12 | 2 | | 100 | 3 | 7 | 17 | |
| 13[37] | 20 | 100 | | 23 | | 30 | |
| 32 | 100 | 18 | 12 | 20 | | 31 | |
| 33 | 100 | 24 | 12 | 30 | | 30 | |
| 1 | 93[a] | | 68 | | | | 55 (100) |
| 9 | 67 | | 12 | 1 | 34 | 8 | 69 (100) |
| 16 | 83 | 1 | 11 | 3 | 53 | 9 | 69 (100) |
| 7 | 15 | 1 | 23 | 1 | 7 | 6 | 69 (100) |
| 26 | 28 | | 33 | 1 | 8 | 5 | 69 (100) |
| 34 | 100[a] | | 38 | | | 96 | |
| 23 | 1 | | 100 | 2 | 3 | 9 | |
| 24 | 6 | | 100 | 3 | 3 | 7 | |
| 29 | 42 | 13 | 22 | 21 | | 100 | |
| 30 | 38 | 14 | 23 | 15 | | 100 | |
| 35 | 29[a] | 1 | | 16 | | 100 | |
| 31 | 53 | 100 | 11 | 29 | | 29 | |

[a] For $m/z$ 384.

300.244 60 (12, $C_{21}H_{32}O$), 273.219 41 (22, $C_{19}H_{29}O$), 272.212 99 (34, $C_{19}H_{28}O$), 255.211 63 (70, $c_{19}H_{27}$), 69.070 36 (100, $C_5H_9$).

**22-Methylcholesta-5,22(*Z*)-dien-3β-ol (16):** mp 147–148 °C (MeOH); $[\alpha]^{20}_D$ −73°; high-resolution mass spectrum; $m/z$ (relative intensity, assignment) 398.354 55 (83, $C_{28}H_{46}O$, $M^+$) 383.330 92 (5, $C_{27}H_{43}O$), 380.341 98 (16, $C_{28}H_{44}$), 342.291 58 (3, $C_{24}H_{38}O$), 300.245 28 (11, $C_{21}H_{32}O$), 273.258 30 (28, $C_{19}H_{29}O$), 272.214 32 (53, $C_{19}H_{28}O$), 255.212 09 (99, $C_{19}H_{27}$), 69.070 41 (100, $C_5H_9$).

**22-Methylcholesta-4,22(*E*)-dien-3β-ol (17):** mp 137–139 °C (MeOH); $[\alpha]^{20}_D$ +4.6°; high-resolution mass spectrum; $m/z$ (relative intensity, assignment) 398.354 06 (16, $C_{28}H_{46}O$, $M^+$), 380.345 86 (47, $C_{28}H_{44}$), 328.314 08 (4, $C_{24}H_{40}$), 273.220 03 (8, $C_{19}H_{29}O$), 255.211 33 (47, $C_{19}H_{27}$), 69.070 51 (100, $C_5H_9$).

**22-Methylcholesta-4,22(*Z*)-dien-3β-ol (18):** mp 135–136 °C (MeOH); $[\alpha]^{20}_D$ +10°; high-resolution mass spectrum; $m/z$ (reltive intensity, assignment) 398.355 78 (20, $C_{28}H_{46}O$, $M^+$), 380.343 78 (100, $C_{28}H_{44}$), 328.315 52 (4, $C_{24}H_{40}$), 273.220 48 (4, $C_{19}H_{29}O$), 255.211 09 (51, $C_{19}H_{27}$), 69.070 40 (51, $C_5H_9$).

**Isomerization of 23-Methylenecholesterol (12).** 23-Methylcholesta-5,22(*E*)-dien-3β-ol (**7**) and 23-methylcholesta-5,23(*E*)-dien-3β-ol (**23**) were obtained in pure form directly by separation on an ODS-2 column. The use of an ODS-3 column with 95% methanol as solvent was effective for separation of 23-methylcholesta-5,22(*Z*)-dien-3β-ol (**26**) and 23-methyl-

cholesta-4,22(*E*)-dien-3β-ol (**25**), since they had same retention time on an ODS-2 column. The pure 23-methylcholesta-5,23(*Z*)-dien-3β-ol (**24**) was obtained by way of 10% $AgNO_3$–silica gel thin-layer chromatography (TLC) of its acetate with benzene/hexane (1:9) as the eluent.

**23-Methylcholesta-5,22(*E*)-dien-3β-ol (7):** mp 155–156 °C (MeOH); $[\alpha]^{20}_D$ −46° (lit.[4] mp 141–143 °C; $[\alpha]^{20}_D$ −36); high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.357 46 (15, $C_{28}H_{46}O$, $M^+$), 380.345 95 (6, $C_{24}H_{44}$), 300.243 46 (23, $C_{21}H_{32}O$), 272.214 49 (7, $C_{19}H_{28}O$), 255.209 88 (24, $C_{19}H_{27}$), 69.070 17 (100, $C_5H_9$).

**23-Methylcholesta-5,22(*Z*)-dien-3β-ol (26):** mp 178–180 °C (MeOH); $[\alpha]^{20}_D$ −44°; high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.356 70 (28, $C_{28}H_{46}O$, $M^+$), 380.343 63 (5, $C_{28}H_{44}$), 300.244 84 (33, $C_{21}H_{32}O$), 272.212 78 (8, $C_{19}H_{28}O$), 255.210 53 (28, $C_{19}H_{27}$), 69.070 24 (100, $C_5H_9$).

**23-Methylcholesta-5,23(*E*)-dien-3β-ol (23):** mp 164–166 °C (MeOH); $[\alpha]^{20}_D$ −41°; high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.352 98 (1, $C_{28}H_{46}O$, $M^+$), 380.345 63 (3, $C_{28}H_{44}$), 300.242 32 (100, $C_{21}H_{32}O$), 285.221 10 (24, $C_{20}H_{29}O$), 283.240 36 (25, $C_{21}H_{31}$), 282.234 19 (25, $C_{21}H_{30}$), 271.206 80 (9, $C_{19}H_{27}O$), 267.211 18 (22, $C_{20}H_{27}$), 255.212 27 (3, $C_{19}H_{27}$).

**23-Methylcholesta-5,23(*Z*)-dien-3β-ol (24):** mp 134–136 °C (MeOH); high-resolution mass spectrum; *m/z* (relative intensity, assignment) 398.352 15 (6, $C_{28}H_{46}O$, $M^+$), 380.341 20 (3, $C_{28}H_{44}$), 300.242 41 (100, $C_{21}H_{32}O$), 285.219 44 (12, $C_{20}H_{29}O$), 283.241 56 (29, $C_{21}H_{31}$), 282.234 11 (11, $C_{21}H_{30}$), 271.205 66 (7, $C_{19}H_{27}O$), 267.210 31 (7, $C_{20}H_{27}$).

**23-Methylcholesta-4,22(*E*)-dien-3β-ol (25):** high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.353 87 (15, $C_{28}H_{46}O$, $M^+$), 380.345 76 (49, $C_{28}H_{44}$), 300.245 72 (10, $C_{21}H_{32}O$), 273.219 70 (5, $C_{19}H_{29}O$), 272.212 07 (5, $C_{19}H_{28}O$), 255.209 28 (28, $C_{19}H_{27}$), 69.070 50 (100, $C_5H_9$).

**Isomerization of 24-Methylenecholesterol (13).** Ergosta-5,23(*E*)-dien-3β-ol (**29**) and ergosta-5,24-dien-3β-ol (**31**) were obtained directly from crude mixture by separation on an ODS-2 column. Since ergosta-5,23(*Z*)-dien-3β-ol (**30**) and epicodisterol (**33**) had the same retention time as 24-methylenecholesterol (**13**) on an ODS-2 column; 10% $AgNO_3$–silica gel TLC (with benzene/hexane (1:9) as the eluent) was used for the separation of these two compounds. Codisterol (**32**) also had to be purified by 10% $AgNO_3$–silica gel TLC of its acetate with benzene hexane (1:9) as the eluent, though its retention time on an ODS-2 column is slightly different from that of **30** and **33**.

**Ergosta-5,23(*E*)-dien-3β-ol (29):**[31] mp 150–151 °C (MeOH); $[\alpha]^{20}_D$ −40.2°; high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.355 62 (42, $C_{28}H_{46}O$, $M^+$), 383.328 46 (7, $C_{27}H_{43}O$), 380.341 08 (6, $C_{28}H_{44}$), 314.261 52 (13, $C_{22}H_{34}O$), 301.253 32 (17, $C_{21}H_{33}O$), 300.247 11 (22, $C_{21}H_{32}O$), 299.238 03 (21, $C_{21}H_{31}O$), 283.241 30 (47, $C_{21}H_{31}$), 271.203 90 (100, $C_{19}H_{27}O$).

**Ergosta-5,23(*Z*)-dien-3β-ol (30):** high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.354 97 (39, $C_{28}H_{46}O$, $M^+$), 383.332 81 (10, $C_{27}H_{43}O$), 380.344 40 (6, $C_{28}H_{44}$), 314.261 24 (14, $C_{22}H_{34}O$), 301.255 10 (23, $C_{21}H_{33}O$), 300.245 67 (23, $C_{21}H_{32}O$), 299.238 37 (15, $C_{21}H_{31}O$), 283.241 36 (57, $C_{21}H_{31}$), 271.205 90 (100, $C_{19}H_{27}O$).

**Ergosta-5,24-dien-3β-ol (31):** mp 141–142 °C (MeOH); $[\alpha]^{20}_D$ −46.7° (lit.[28] mp 141.5–142.5 °C; $[\alpha]_D$ −36.1°) high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.353 25 (53, $C_{28}H_{46}O$, $M^+$), 383.330 19 (11, $C_{27}H_{43}O$), 380.345 26 (2, $C_{28}H_{44}$), 314.258 41 (100, $C_{22}H_{34}O$), 300.242 24 (11, $C_{21}H_{32}O$), 299.235 35 (29, $C_{21}H_{31}O$), 281.225 43 (18, $C_{21}H_{29}$), 271.206 21 (29, $C_{19}H_{27}O$).

**Codisterol (32):** high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.356 00 (100, $C_{28}H_{46}O$, $M^+$), 383.330 08 (13, $C_{27}H_{43}O$), 380.343 90 (13, $C_{28}H_{44}$), 365.322 52 (15, $C_{27}H_{41}$), 328.275 84 (6, $C_{23}H_{36}O$), 314.259 19 (18, $C_{22}H_{34}O$), 300.243 69 (12, $C_{21}H_{32}O$), 299.236 80 (19, $C_{21}H_{31}O$), 271.205 02 (31, $C_{19}H_{27}O$).

**Epicodisterol (33):** high-resolution mass spectrum, *m/z* (relative intensity, assignment) 398.354 61 (100, $C_{28}H_{46}O$, $M^+$), 383.333 63 (13, $C_{27}H_{43}O$), 380.342 80 (21, $C_{28}H_{44}$), 365.322 12 (18, $C_{27}H_{41}$), 328.277 45 (8, $C_{23}H_{36}O$), 314.260 02 (24, $C_{22}H_{34}O$), 300.242 54 (12, $C_{21}H_{32}O$), 299.238 99 (31, $C_{21}H_{31}O$), 271.206 75 (30, $C_{19}H_{27}O$).

**Isomerization of Cholesterol (21).** Cholesterol (**21**) was subjected to reaction with the *N*-lithioethylenediamine reagent for 40 h. After the crude mixture was separated on an ODS-2 column, aside from 81% of starting material, there was obtained 19% of cholest-4-en-3β-ol (**22**).

**Cholest-4-en-3β-ol (22):** mp 130–131 °C (EtOAc); (lit.[25] mp 131–132 °C); $^1H$ NMR 0.675 (3 H, s, 18-$CH_3$), 1.047 (3 H, s, 19-$CH_3$), 0.899 (3 H, d, $J$ = 6.56 Hz, 21-$CH_3$), 0.859 (3 H, d, $J$ = 6.64 Hz, 26-$CH_3$), 0.863 (3 H, d, $J$ = 6.62 Hz, 27-$CH_3$), 4.147 (1 H, m, 3-CH), 5.271 (1 H, m, 4-CH).

**Isomerization of Dinosterol (11).** Dinosterol (**11**)[39] was subjected to isomerization for 40 h as described above, and the crude product was separated on an ODS-2 column; aside from 74% of starting material, there was obtained 18% of the 24β-methyl isomer **28**, which was characterized by its $^1H$ NMR data, which were the same as those of the synthetic 24β-methyl isomer **28**:[27] $^1H$ NMR 0.683 (3 H, s, 18-$CH_3$), 0.827 (3 H, s, 19-$CH_3$), 0.910 (3 H, d, $J$ = 6.72 Hz), and 0.937 (3 H, d, $J$ = 6.75 Hz) for 21-$CH_3$ and 24-$CH_3$, 1.485 (3 H, s, 23-$CH_3$), 0.771 (3 H, d, $J$ = 6.72 Hz, 26-$CH_3$), 0.849 (3 H, d, $J$ = 6.34 Hz, 27-$CH_3$), 0.946 (3 H, d, $J$ = 6.12 Hz, 4-$CH_3$), 4.875 (1 H, d, $J$ = 9.36, 22-CH).

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