Synthesis of (24*R*,28*R*)- and (24*S*,28*S*)-24,28-Methylene-5-stigmasten-3β-ol and Biosynthetic Implications of Cyclopropyl Cleavage to 24-Substituted Cholesterols

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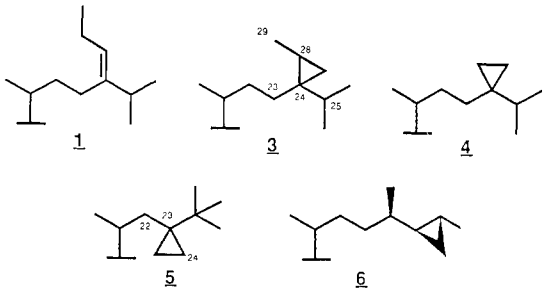
The 24*R*,28*R* and 24*S*,28*S* isomers (**3c,d**) of 24,28-methylene-5-stigmasten-3β-ol (**3**), a potential biosynthetic precursor of 24-propylidenecholesterol (**1**) via cyclopropyl ring opening, were synthesized and assigned absolute stereochemistry. A highly stereospecific acid-catalyzed cyclopropyl ring opening was demonstrated with C-29 ^{13}C-labeled sterols. The acid-catalyzed ring openings of model cyclopropyl sterols **4** and **5** were investigated by using ^{13}C- and ^{2}H-labeled sterols. Kinetic rate constants and deuterium isotope effects of cyclopropane and olefin isomerizations were determined. A mechanism via a classical carbonium ion was proposed for the ring opening of cyclopropyl sterol **5**. Implications of acid-catalyzed cyclopropyl ring opening are discussed in terms of the intermediacy of protonated cyclopropanes in the biosynthesis of marine sterols.

The isolation of (24*E*)-24-propylidenecholesterol (**1**) from Chrysophyte algae of the order Sarcinochrysidales[1] has led us to study the biosynthetic mechanism of side chain alkylation in a Chrysophyte algae of this order.[2] Although 24-vinylcholesterol[3] (**2**) is a logical precursor (Figure 1, scheme 1), it cannot be detected even in trace amounts. However, two isomers of 24,28-methylene-5-stigmasten-3β-ol (**3**)—(24*S*,28*R*)-24,28-methylene-5-stigmasten-3β-ol (**3b**) and an unidentified diastereomer—were found to the extent of 0.07% of the isolated sterols. Also detected in trace quantities in this alga were sterols **7–10**, which could also conceivably arise via cyclopropyl ring opening of **3** (Figure 1, scheme 2). In several other marine organisms



steroidal cyclopropanes and olefins that can conceivably arise from cyclopropyl ring opening also co-occur.[4] We have considered the possibility of enzyme-mediated acid-catalyzed ring openings as biosynthetic reactions in marine organisms[5] by analogy to the cycloartenol ring opening in higher plants.[6] When the Chrysophyte alga was grown in the presence of deuterium-labeled methionine, the incorporation of six deuterium atoms into 24-propylidenecholesterol (d_6-**1**, Figure 1, scheme 3) ruled out an acid-catalyzed ring opening of **3** since this route should lead to the incorporation of five deuteriums (d_5-**1**).[2] However, it is conceivable that a protonated cyclopropane is produced directly as a high-energy intermediate from fucosterol (**11a**) or isofucosterol (**11b**) by the enzymatic sterol-*S*-adenosyl-methionine methyl transfer reaction.[5e,7] In this case the deuterionated cyclopropane could yield **1** with the observed degree of deuterium substitution. Alternative ring openings of this protonated species could lead to the trace sterols **7–10**, and simple deprotonation would give rise to the cyclopropane **3**.

In this paper we describe the synthesis of all four stereoisomers of the putative cyclopropane biosynthetic intermediate **3** and a mechanistic study of its nonenzymatic acid-catalyzed ring opening with ^{13}C-labeled compounds. The mechanism of nonenzymatic acid-catalyzed cyclopropane ring openings in the sterol side chain was further explored with model compounds **4** and **5** by using kinetic

Table I. Methyl Group Region ^1H NMR (300 MHz, $CDCl_3$) Chemical Shifts

	chemical shift[a]				
compd	C18	C19	C21	C26,27	C29
3a	0.672	1.005	0.917 (6.45)	0.831, 0.805 (7.07, 7.05)	1.032 (6.28)
3b	0.673	1.007	0.915 (6.49)	0.833, 0.827 (6.85, 6.66)	1.044 (6.27)
3c	0.660	1.003	0.878 (6.24)	0.981, 0.954 (7.33, 7.28)	1.074 (6.36)
3d	0.663	1.005	0.881 (6.45)	0.985, 0.956 (7.10, 6.96)	1.081 (6.26)

[a] Given as δ values; *J* values given in parentheses in hertz.

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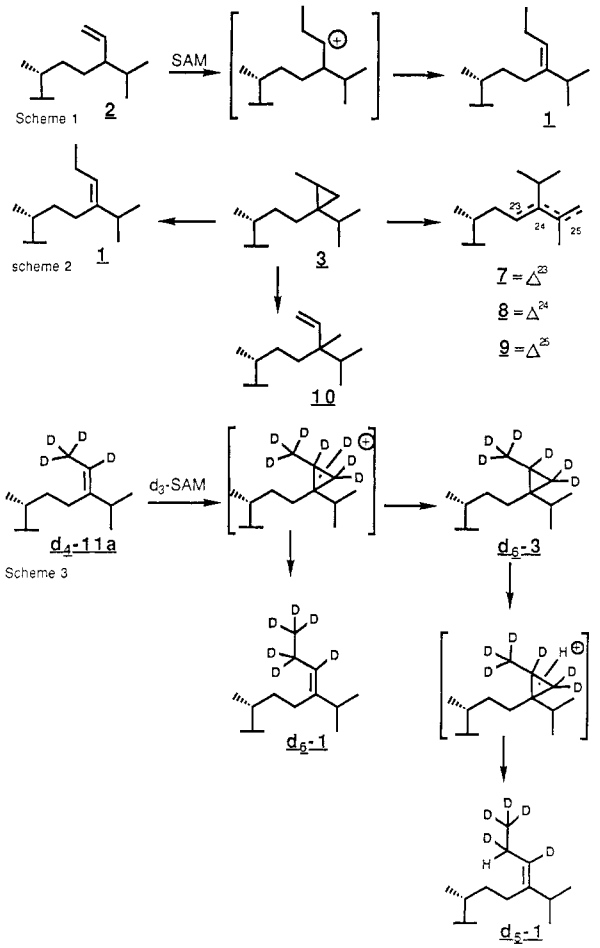

Figure 1. Possible biosynthetic routes to 24-propylidenecholesterol (1).

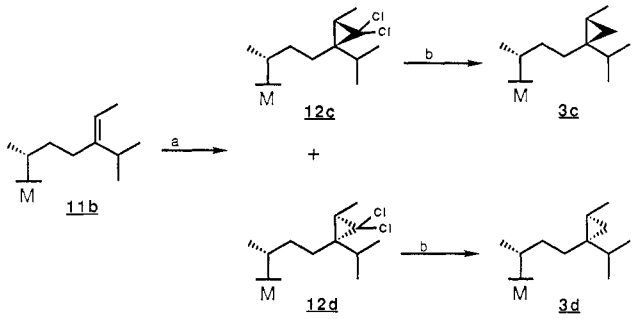

Figure 2. Synthesis of 24,28-methylenestigmasterol diastereomers (3c,d). (a) $CHCl_3/NaOH$; (b) Li/NH_3.

measurements and isotopic labeling experiments.

Preparation of 24,28-Methylene-5-stigmasten-3β-ol Isomers and Assignment of Absolute Stereochemistry. The two undescribed isomers of **3** were synthesized from isofucosterol (**11b**) by the route previously described for the fucosterol (**11a**) derived diastereomers (**3a,3b**)[8] (Figure 2 and Table I). Comparison of the reductive ring opening products (**13a,b**) of all four isomers of **3** (Figure 3, scheme 1) led to assignment of the absolute stereochemistries of **3c** and **3d** through correlation with the absolute stereochemistries of the fucosterol-derived isomers previously determined by X-ray analysis.[9] This allows us to report that the unidentified naturally occurring isomer

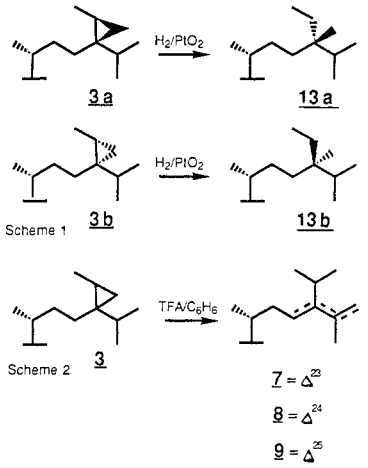

Figure 3. Ring-opening reactions of 3.

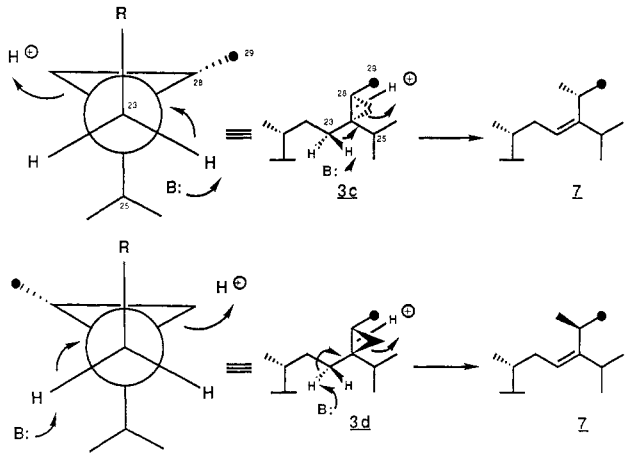

Figure 4. Possible E2-like concerted acid-catalyzed ring openings of [29-^{13}C]-**3c** and -**3d** (shown for the major conformers) (●, ^{13}C).

of **3** isolated from the Chrysophyte[2] is the 24*R*,28*R* isomer **3d**. Furthermore, reductive cleavage of chiral 24,28-methylenestigmastane side chains (**3**) leads to the stereospecific synthesis of the 24-methyl-24-ethyl side chain (**13**), which has been synthesized and isolated[10] (also in unsaturated form **10**[2]) but has escaped chiral assignment.

Acid-Catalyzed Cyclopropane Ring Opening Products and Mechanistic Studies. Acid-catalyzed ring openings of many natural marine sterols, such as petrosterol (**6**), as well as synthetic analogues have been studied in our laboratory.[5c,d,11] In these studies the stereochemistry of the olefinic products indicated that the ring-opening reactions proceeded via concerted mechanisms in which an antiperiplanar relationship exists between the breaking cyclopropyl bond and the departing proton.[5c,d,11a-e] We have used these mechanistic considerations to assign the stereochemistry of several marine sterols.[5d,11a-c]

When each of the four isomers of 24,28-methylene-5-stigmastenol (**3**) was subjected to acid-catalyzed ring opening in 5% TFA/C_6H_6, the same product distribution was observed for each isomer: **7** (15%), **8** (47%), **9** (38%) (Figure 3, scheme 2).[1a,10,12] Neither 24-propylidenecholesterol (**1**) nor **10** was detected. This outcome is in agree-

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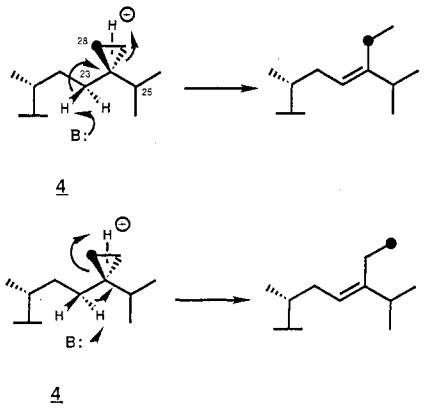

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Figure 5. Possible E2-like concerted acid-catalyzed ring openings of 24*R* [28-^{13}C]-4 (shown for the major conformer) (●, ^{13}C).

ment with the rule[13] that the bond between the most substituted and the least substituted carbons is usually broken. Observation of a stereospecific ring opening is, in this case, obscured by the symmetry of the products. In order to determine if a stereospecific ring opening occurs, all isomers of **3** were synthesized with ^{13}C in the C-29 position. The products of the ring opening of **3** were also synthesized in ^{13}C-labeled form for use as ^{13}C NMR reference compounds. If the ring-opening reaction proceeded via a concerted E2-like mechanism, the stereochemical course of such a ring opening would dictate that starting from a pure diastereomer of **3**, labeled with ^{13}C in the 29-position, only one of the four methyl groups will bear the label in the product (Figure 4).

An attempt to monitor the ring-opening reaction of [29-^{13}C]-**3a** by ^{13}C NMR in 10% TFA-*d*/C_6D_6 resulted in extensive deuterium exchange. Subsequent kinetic rate measurements showed the ring-opening reaction ($t_{1/2}$ 58 min) to be slower than the acid-catalyzed isomerization of the olefinic products (**7**, $t_{1/2}$ 42 min; **8**, $t_{1/2}$ 10.5 min; **9**, $t_{1/2}$ 13.5 min). However, the rate of isomerization of **7** is sufficiently slow, in relation to the rate of the ring opening, that the initial product of ring opening should be detectable early in the course of the reaction before subsequent scrambling of the label. When [29-^{13}C]-**3c** was treated with 10% TFA/C_6H_6 for 1 h, 60% of the ^{13}C label was found in one of the four possible positions of the isolated product **7**. When the experiment was repeated with [29-^{13}C]-**3d**, 60% of the ^{13}C label in **7** was found mainly in one of the other positions. The stereospecificity observed in this ring opening prior to secondary isomerization is suggestive of a concerted mechanism such as the E2-like mechanism in Figure 4. However, in the absence of knowledge of the absolute stereochemistry of the ^{13}C-labeled products or of experiments utilizing substrates stereospecifically labeled with deuterium at C-23, and antiperiplanar relationship between the breaking cyclopropane bond and the departing proton at C-23 cannot be proven. Also, because the isomerization of **8** is rapid compared to the ring-opening reaction, it is impossible to determine whether a similar stereoselective ring opening takes place, leading to **8** through the removal of the proton at C-25.

In order to explore further the mechanisms of cyclopropane ring openings in sterol side chains, the model compound **4** was prepared. If protonated cyclopropanes exist as intermediates in the SAM biomethylation of sterol side chains, **4** could conceivably be the nonprotonated form of the intermediate in the biosynthesis of 24-ethyl sterols from 24-methylenecholesterol. Although the rates of isomerization of the 24-ethyl sterols produced from ring opening ($t_{1/2}$ < 15 min) are very fast compared to the ring opening itself ($t_{1/2}$ 180 min), substitution of ^{13}C at either of the diastereomeric cyclopropane carbons makes the observation of the stereochemistry of the cyclopropane ring opening step possible. The selective cleavage of one of the two bonds might be favored in the event of an E2-like concerted ring opening (Figure 5) if one of the diasterotopic protons at C-23 is more accessible to base than the other.

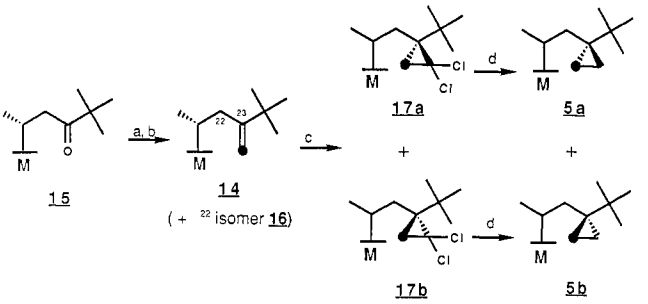

Figure 6. Synthesis of diastereomeric C-24 ^{13}C-labeled *tert*-butylcyclopropyl sterols **5a** and **5b**. (a) [^{13}C]MeMgI; (b) $POCl_3$/pyridine; (c) $CHCl_3$/NaOH; (d) Li/NH_3 (●, ^{13}C).

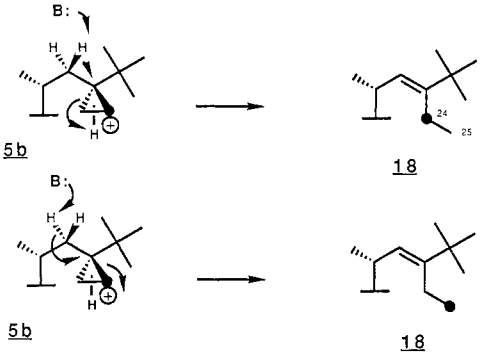

Figure 7. Possible E2-like concerted acid-catalyzed ring openings of 23*R* C-24 ^{13}C-labeled *tert*-butylcyclopropane **5b** (shown for the major conformer) (●, ^{13}C).

The model cyclopropyl sterol (**4**) was prepared with the ^{13}C label in either the 24*R* or 24*S* form by separating the diastereomeric dichlorocyclopropanes by HPLC and removing the chlorine atoms by Birch reduction. The products of the TFA ring openings of both of the diastereomerically ^{13}C-labeled forms of **4** were isolated, and the ^{13}C NMR spectra were measured. The main products of the ring openings of the two sterols showed very little difference (1.10 vs 0.99) in the ratio of the integrals of the C-28 and C-29 labeled carbons. Treatment of [29-^{13}C]-fucosterol (**11a**) under the same conditions showed that no exchange was possible between C-28 and C-29. Whether this reaction proceeds through a stereospecific or a nonstereospecific mechanism cannot be determined. A concerted E2-like mechanism as shown in Figure 5 would require that the protons at C-23 be sterically distinguishable by the base. This is unlikely, however, since they are two bonds removed from the chiral center at C-20. Furthermore, as in the case of **3**, removal of the proton at C-25 may also lead to product, whereupon no selectivity in the bond fission would be expected.

Therefore, still another model compound (**5**) was constructed (Figure 6). In this case only the removal of the diastereotopic protons at C-22 can lead to product via an E2-like concerted process (Figure 7). These protons should have very different steric accessibilities due to their

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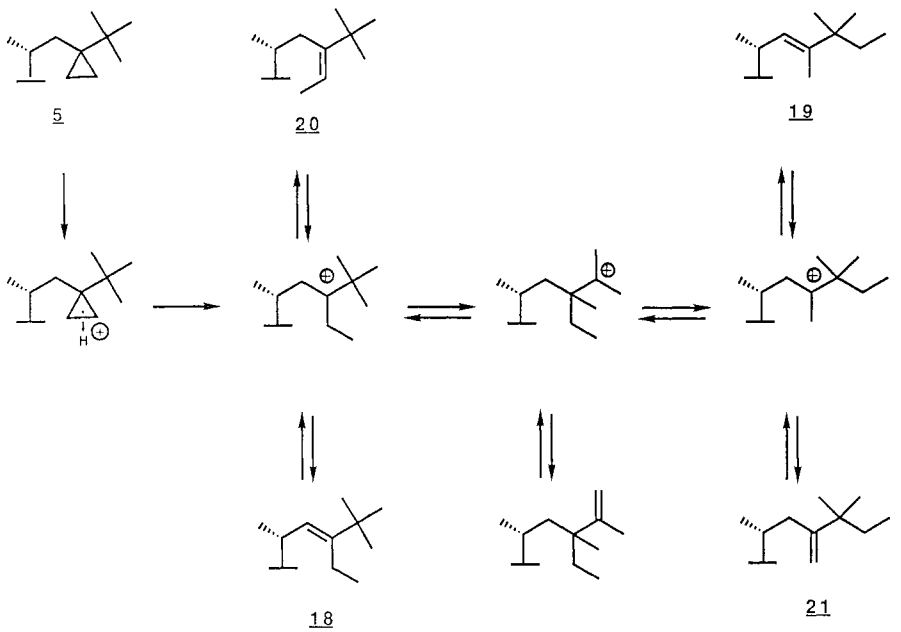

Figure 8. Acid-catalyzed isomerization of *tert*-butylcyclopropyl sterol **5** and its isomerization products (**18**, **19**, and **20**) via carbonium ions.

proximity to the bulky sterol nucleus. Because of steric hindrance the olefinic intermediate **14** could not be obtained from the ketone **15** via a Wittig reaction. Reaction of the ketone with methylmagnesium iodide followed by $POCl_3$ dehydration gave a 1:2 mixture of the olefins **14** and **16**, which were separated by $AgNO_3$–silica gel chromatography. Treatment of **14** with dichlorocarbene gave a 9:1 mixture of the two diastereomeric dichlorocyclopropanes (**17a,b**). The ^{13}C NMR spectrum of the major isomer (**17b**) showed considerable line broadening due to hindered rotation. The ^1H NMR spectrum also showed broadening of the C-21 methyl signal. Separation and reduction of the dichlorocyclopropanes gave the diastereomerically ^{13}C-labeled cyclopropyl sterols (**5a,b**). Three products were isolated after TFA ring opening. These were determined to be **18**, **19**, and **20** by NMR, MS, and synthesis (Figure 8). The ratios of the methyl (C-25) and methylene (C-24) ^{13}C NMR integrals differed for the ring-opening product **18** (Figure 7) of the two cyclopropanes **5a** and **5b**. However, the differences were small and no differences in the [^{13}C]methyl/methylene ratios arising from the two diastereomerically labeled [^{13}C]-cyclopropanes were noted in the rearranged product **10**. This indicates either a nonstereoselective mechanism or, the less likely possibly, that there is no discrimination in the abstraction of the C-22 protons in a stereoselective mechanism.

Kinetic measurements were made of the isomerizations of the three olefins and the cyclopropane. The cyclopropyl ring opening ($t_{1/2}$ 280 min) and the Wagner–Meerwein isomerization of **18** to **19** ($t_{1/2}$ 1370 min), which accounts for 96% of the equilibrium mixture, were slow compared to the isomerization of **20** ($t_{1/2}$ 65 min). In an E2-like ring opening, **18** is the expected initial product; yet when **18** is isomerized no **20** can be detected. When the ring opening was carried out in TFA-*d*/C_6D_6, a k_H/k_D of 3.4 was measured. When starting material was recovered after the reaction was half completed, no deuterium exchange could be detected by mass spectrometry. This is in agreement with Wiberg's results[14] and indicates that the initial protonation is rate determining. The same pattern was seen in the isomerization of **20** (k_H/k_D = 2.0) and the Wagner–Meerwein rearrangement of **18** to **19** (k_H/k_D = 1.7). When the hydrogens at C-22 were replaced with deuterium, no isotope effect was observed. Isolation of **20** formed early in the ring-opening reaction showed that both of the C-22 deuteriums had been retained during the ring opening, thereby ruling out a E2-like mechanism in the formation of this product and providing evidence for a classical carbonium ion intermediate (Figure 8).

Summary and Biosynthetic Implications. All isomers of **3**, a potential biosynthetic intermediate of 24-propylidenecholesterol (**1**) in a marine Chrysophyte alga, were prepared, and the absolute stereochemistries were determined. The mechanism of nonenzymatic acid-catalyzed ring opening of these cyclopropyl sterols, as well as the model cyclopropanes **4** and **5**, was studied with isotopic labeling and kinetic experiments.

Labeling with carbon-13 provided a method by which a stereospecific ring opening could be detected for **3** despite the high degree of symmetry of the products. However, we cannot rule out the possibility of additional competing pathways that were not detected owing to isomerization of the products. The isomerization of the products under the reaction conditions is a problem that makes it very difficult to study cyclopropane ring openings that proceed at comparatively slow rates, e.g. the ring opening of **4** is 15 times slower than that for the isomerization of its products. Stereospecific ring openings involving antiperiplanar relationships between the breaking cyclopropyl bond and the departing proton have been demonstrated in the case of several cyclopropyl sterols and have provided the basis for a proposed biosynthetic scheme for the origin of several marine sterols.[5e] This is the first stereospecific acid-catalyzed cyclopropane ring opening that we have demonstrated involving what could formally be considered a tertiary carbonium ion.

In the case of the model cyclopropyl sterol **5**, deuterium labeling showed that ring opening can take place with the loss of a proton from the cyclopropyl ring itself. This result can be explained by a mechanism involving a classical carbonium ion intermediate. A route via a classical tertiary carbonium ion may be favorable due to the high degree of steric congestion that was built into this model compound. On the other hand, several mechanisms may be

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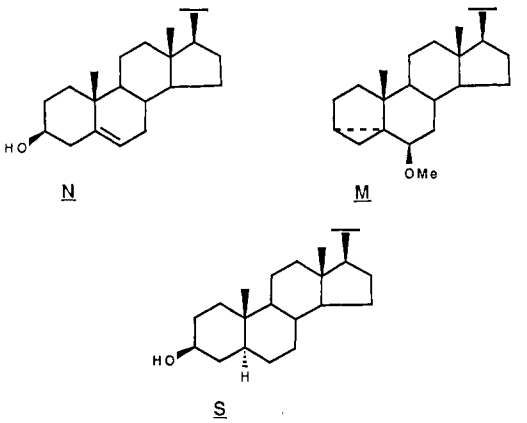
co-occurring in the ring opening of this cyclopropyl sterol, but only the one involving loss of a proton from the cyclopropane ring could be demonstrated due to experimental limitations. A multiplicity of concerted mechanisms have been demonstrated for the acid-catalyzed ring opening of the marine sterol petrosterol (**6**) leading to products via 1,2-hydride shifts, 1,3-hydride shifts, 1,5-hydride shifts, and 1,2-methyl shifts in addition to an E2-like process.[11e] This is the first time evidence for ring opening via a classical carbonium ion has been observed in sterols bearing a cyclopropyl side chain, although a nonconcerted mechanism of ring opening, perhaps involving a classical carbonium ion, has been considered in the enzymatic isomerization of cycloeucalenol.[6]

We have established that the naturally occurring sterols **1** and **7** are not formed from the ring opening of **3** under our conditions. However, considering the importance of geometry and the placement of the base in the transition state, it is conceivable that, in the rigidly defined context of an enzyme, the ring opening of the intermediate protonated cyclopropane may occur by an alternative pathway. The ring opening of **5**, involving the loss of a proton from the cyclopropane ring itself, provides a precedent for the possible ring opening of **3** leading to **1**. It is interesting to note that both of the isomers of **3** found in the Chrysophyte have the cyclopropyl methylene group in the β orientation. This suggests that both are synthesized by one-carbon transfer to fucosterol (**11a**) and isofucosterol (**11b**) at a single enzyme that binds both isomeric sterols. The hypothetical enzymatic ring opening of the cyclopropane can likewise occur at a single enzyme that binds both isomers and stereospecifically catalyzes the ring opening to yield one double bond isomer (*E*) of 24-propylidenecholesterol (**1**). If a protonated cyclopropane intermediate is involved in the methyltransfer reaction, both the methyltransfer and the cyclopropyl ring opening may occur at the same enzyme. Biosynthetic experiments to test the hypothetical intermediacy of cyclopropyl sterols in the biosynthesis of 24-propylidenecholesterol (**1**) are difficult because the alga does not take up radiolabeled sterols from the culture medium. Experiments using cell-free extracts are in progress.

Experimental Section

General Methods. High-pressure liquid chromatography (HPLC) was carried out with use of differential refractometric detection and two Altex Ultrasphere ODS 5-μm columns (10 mm i.d. × 25 cm) in series with either methanol (MeOH) or acetonitrile/methanol/ethyl acetate, 3:1:1 (MeCN/MeOH/EtOAc), as the mobile phase. Cholesterol was used as a standard for relative retention times (RRT) for HPLC and GC. Analytical GC (flame-ionization detector) was performed with a U-shaped glass column (2 mm i.d. × 1.8 m) packed with 3% SP 2250 or a SE-54 coated fused silica capillary column (0.32 mm i.d. × 15 m). GC/MS analyses were conducted with the latter column. GC RRTs were determined on the first of these columns. Melting points are uncorrected. All NMR spectra are referenced to the solvent peak ($CHCl_3$ or C_6H_6). The different sterol nuclei are indicated by the suffixes N, M, and S. Thus N refers to the normal Δ^5-3β-hydroxy nucleus, M to the *i*-methyl ether, and S to the saturated 5α-3β-hydroxy nucleus.

Synthesis of 24,28-Methylene-5-stigmasten-3β-ols. A solution of 52.6 mg of isofucosterol *i*-methyl ether (**11b-M**) (from fucosterol (**11a-N**) via the epoxide according to the method of Nicotra and Toma[15]) in 4 mL of chloroform was treated with 26 mg of benzyltriethylammonium chloride and 3 mL of 50% aqueous sodium hydroxide at 0 °C for 16 h under Ar. The mixture was extracted with water and methylene chloride. The combined organic fraction was dried (Na_2SO_4), concentrated, and filtered through silica gel (hexane/ethyl acetate, 9:1) to give, after evaporation of solvent and separation by reverse-phase HPLC (MeOH), two dichlorocyclopropanes (**12c-M** and **12d-M**).



N M S

Fraction A. (24*R*,28*S*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12c-M): 21.5 mg (34%); mp 123–126 °C (MeOH); HPLC (Altex) RT 100 min (3 mL of MeOH/min); ^1H NMR (300 MHz) δ 3.323 (s, 3 H, OCH_3), 1.166 (d, *J* = 6.4 Hz, 3 H, C29), 1.06 (d, *J* = 6.4 Hz, 3 H, C21), 1.026 (s, 3 H, C19), 0.940 (d, *J* = 6.4 Hz, 3 H) and 0.933 (d, *J* = 6.4 Hz, 3 H) C26 and C27, 0.717 (s, 3 H, C18); MS, *m/z* (relative intensity) 510 (0.6, M + 2), 509 (0.3, M + 1), 508 (1.2, M^+), 493 (3), 478 (1), 476 (2), 455 (4), 453 (7), 399 (5), 398 (16), 383 (20), 343 (12), 286 (21), 285 (86).

Fraction B. (24*S*,28*R*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12d-M): 26 mg (41%); mp 41–45 °C (MeOH); HPLC (Altex) RT 101 min; ^1H NMR (300 MHz) δ 3.324 (s, 3 H, OCH_3), 1.165 (d, *J* = 6.4 Hz, 3 H, C29), 1.020 (s, 3 H, C19), 0.942 (d, *J* = 6.4 Hz, 3 H) and 0.937 (d, *J* = 7.01 Hz, 3 H) C26 and C27, 0.708 (s, 3 H, C18); MS, *m/z* (relative intensity) 510 (1.1, M + 2), 508 (1.6, M), 493 (4), 476 (2), 453 (8), 398 (21), 383 (25), 366 (14), 351 (7), 343 (26), 314 (19), 227 (20), 93 (70), 55 (100).

(24*R*,28*R*)-24,28-Methylenestigmast-5-en-3β-ol (3c): Dehalogenation of the dichloro-*i*-methyl ether **12c-M** was accomplished with lithium in liquid ammonia according to a modified literature procedure:[11f] lithium (40 mg) was added to 50 mL of condensed ammonia, and the resulting blue solution was allowed to stir for 1 h. The dichloro-*i*-methyl ether **12c-M** (21.5 mg, 0.04 mmol) in 3 mL of dry ether was added to the reaction, and after 2 h the reaction was quenched by the addition of 10 mL of ether/ethanol (1:1), and the ammonia was allowed to evaporate. The mixture was extracted with water and methylene chloride. The combined organic fraction was then washed with brine, dried and evaporated to give the crude steroidal *i*-methyl ether. Recovery of the Δ^5 system was accomplished by heating the *i*-methyl ether under reflux in a mixture of dioxane (1 mL), water (0.5 mL), and toluenesulfonic acid (2.5 mg) until GLC monitoring showed a total loss of starting material (100 min). The reaction was diluted with ether, and the organic layer was washed with brine, dried (Na_2SO_4), concentrated, and filtered through silica gel to yield 10.3 mg (60%) of the cyclopropyl sterol **3c-N**): mp 118–121 °C; for ^1H NMR data see Table I; HRMS, *m/z* (relative intensity) 426.3862 (M^+, 10, calcd for $C_{30}H_{50}O$ 426.3862), 408.3750 ($C_{30}H_{48}$, 4, $M^+ - H_2O$), 393.3503 ($C_{29}H_{45}$, 3, $M^+ - H_2O - CH_3$), 365.3162 ($C_{26}H_{40}$, 3, $M^+ - C_3H_7 - H_2O$), 314.2624 ($C_{22}H_{34}O$, 8), 299.2387 ($C_{21}H_{31}O$, 10), 281.2278 ($C_{21}H_{29}$, 6), 271.2060 ($C_{19}H_{27}O$, 10), 255.2094 ($C_{19}H_{27}$, 5), 213.1641 ($C_{16}H_{21}$, 11), 69.0702 (C_5H_9, 95); MS, *m/z* (relative intensity) 426 (79.5, M^+), 393 (16), 314 (54), 299 (36), 271 (50), 255 (18), 213 (38), 69 (100).

(24*S*,28*S*)-24,28-Methylenestigmast-5-en-3β-ol (3d). Compound **12d-M** was treated in the manner described for the conversion of **12c-M** to **3c-N** to give cyclopropyl sterol **3d-N**. The 3β-hydroxy-Δ^5 system was regenerated as before but for only 100 min (19% completion) due to the instability of the cyclopropane to these conditions: mp 118–120 °C; for ^1H NMR data see Table I; HRMS, *m/z* (relative intensity) 426.3859 (M^+, 20 calcd for $C_{30}H_{50}O$ 426.3862), 408.3767 ($C_{30}H_{48}$, 7), 393.3511 ($C_{29}H_{45}$, 6), 365.3203 ($C_{27}H_{41}$, 4), 314.2614 ($C_{22}H_{34}O$, 20), 299.2364 ($C_{21}H_{31}O$, 15), 281.2269 ($C_{21}H_{29}$, 6), 271.2064 ($C_{19}H_{27}O$, 18), 255.2122

(15) Nicotra, F.; Toma, L. *Gazz. Chim. Ital.* **1980**, *110*, 579–580.

($C_{19}H_{27}O$, 18), 255.2122 ($C_{19}H_{27}$, 7), 213.1653 ($C_{16}H_{21}$, 15), 69.0705 (95).

Cyclopropane Ring Opening of 24,28-Methylene-5-stigmasten-3β-ols (3a–d): Acid-Catalyzed Ring Opening.[11d] The steroidal cyclopropane (0.5 mg, 0.001 mmol) was treated with 1 mL of 5% TFA/C_6H_6 at 25 °C for 2–3 h. The solvent and acid were removed under reduced pressure, and the crude material was filtered through silica gel in ether. The products were separated by reverse-phase HPLC (MeOH) into three fractions (A, B, C). Fraction B was separated by HPLC (MeCN/MeOH/EtOAc) into two fractions (B1 and B2). All four isomers of **3** gave the same product mixture. The ^1H NMR (300 MHz) and mass spectra of the products were in agreement with published values.[2,12c]

Fraction A. (24*R*)-28-Methylstigmasta-5,25-dien-3β-ol acetate (9a):[12c] 15%; HPLC RT 112 min, GLC RRT 2.6.

Fraction B: 37%, HPLC RT 115 min (MeOH). This fraction was further chromatographed to give:

Fraction B1. (24*S*)-28-Methylstigmasta-5,25-dien-3β-ol acetate (9b):[13c] 21%; HPLC RT 90 min (CH_3CN/MeOH/EtOAc), GLC RRT 2.4.

Fraction B2. 28-Methylstigmasta-5,23-dien-3β-ol acetate (7):[2] 15%; HPLC RT 92 min, GLC RRT 2.2.

Fraction C. 28-Methylstigmasta-5,24-cholestadien-3β-ol acetate (8):[2] 48%; HPLC RT 127 min, GLC RRT 3.0.

Reductive Cleavage[16] of 24,28-Methylene-5-stigmasten-3β-ols (3a–d). A solution of the steroidal cyclopropane (1 mg) in 5% acetic acid/methanol (2 mL) was stirred with 1 mg of PtO_2 under a hydrogen balloon for 2–4 days. At this time the reaction was 10–15% complete as shown by GC. The solvent was evaporated, and the residue taken up in methylene chloride and filtered through silica gel. The products were separated by reverse-phase HPLC (MeOH).

From (24*R*,28*S*)-24,28-methylene-5-stigmasten-3β-ol (**3a-N**),[9] **(24*S*)-24-methyl-5α-stigmastan-3β-ol (13a-S)**:[10] 11%; HPLC RRT 1.6, GC RRT 2.2; ^1H NMR (300 MHz) δ 3.60 (m, 1 H, C3), 0.905 (d, J = 7 Hz, 3 H, C21), 0.800 (s, 3 H, C19), 0.789 (d, J = 6.8 Hz, 6 H, C26 and 27), 0.748 (t, J = 7 Hz, 3 H, C29), 0.667 (s, 3 H, C18), 0.644 (s, 3 H, C30); MS, m/z (relative intensity) 430 (M^+), 397 (5), 387 (10), 386 (10), 369 (20), 316 (5), 302 (10), 273 (10), 257 (5), 243 (10), 57 (100); HRMS 430.4171 (calcd 430.4177).

From (24*S*,28*R*)-24,28-methylene-5-stigmasten-3β-ol (**3b-N**),[2,9] **(24*R*)-24-methyl-5α-stigmastan-3β-ol (13b-S)**:[10] 4%; HPLC RRT 1.6, GC RRT 2.2; ^1H NMR (300 MHz) δ 3.58 (m, 1 H, C3), 0.904 (d, J = 6.45 Hz, 3 H, C21), 0.801 (s, 3 H, C19), 0.791 (d, J = 6 Hz, 3 H, C26 and 27), 0.744 (t, J = 8 Hz, 3 H, C29), 0.668 (s, 3 H, C18), 0.643 (s, 3 H, C30); MS, m/z (relative intensity) 430 (16, M^+), 387 (23), 386 (20), 369 (40), 316 (27), 302 (8), 273 (28), 257 (14), 243 (28), 215 (100).

From (24*R*,28*R*)-24,28-methylene-5-stigmasten-3β-ol (**3c-N**), **(24*S*)-24-methyl-5α-stigmastan-3β-ol (13a-S)**: 5%; ^1H NMR and mass spectra same as reported above.

From (24*S*,28*S*)-24,28-methylene-5-stigmasten-3β-ol (**3d-N**): **(24*R*)-24-methyl-5α-stigmastan-3β-ol (13b-S)**: 19%; ^1H NMR and mass spectra same as reported above.

Kinetics of Acid-Catalyzed Cyclopropane Ring Opening and Olefin Isomerization. Free sterols and their *i*-methyl ethers were used interchangeably. Both forms are rapidly converted to the trifluoroacetate esters under the reaction conditions. ^{13}C NMR and GC analyses were used interchangeably.

(a) ^{13}C NMR Analysis. Solutions of the ^{13}C-labeled sterols in 10% TFA/C_6H_6 were prepared in NMR tubes. The reaction was followed by ^{13}C NMR (400 MHz) with an external D_2O reference for the lock signal. For deuterium isotope effects the spectra were measured in the same way with the substitution of TFA-*d*/C_6D_6 and omission of the external reference. Disappearance of sterol was determined by the ratio of the integrals of the sterol and the solvent peaks. Good agreement with pseudo-first-order kinetics was observed.

(b) GC Analysis. Solutions of the ^{13}C-labeled sterols in 10% TFA/C_6H_6 were prepared. At intervals, aliquots of the reaction mixture were removed and hydrolyzed with 5% KOH/MeOH. Extraction with ether and water was followed by purification of the sterols by TLC (eluent hexanes/ether, 1:1). The sterol mixtures were analyzed by capillary GC, and the integrals were used to determine the rates of reaction.

Synthesis of ^{13}C-Labeled 24,28-Methylene-5-stigmasten-3β-ols: [29-^{13}C]-6β-Methoxy-3α,5-cyclo-5α-stigmastan-28-ol.[17] To a solution of 46.3 mg of 24-formylcholesterol *i*-methyl ether[2] in dry ether was added an ether solution of [^{13}C]methylmagnesium iodide (from 0.3 g of [^{13}C]methyl iodide (99.9%) and 100 mg of magnesium). After 10 min at 25 °C the mixture was poured into water and extracted with ether. The organic layer was dried and evaporated. Separation of the four isomers by preparative TLC allowed measurement of the ^{13}C NMR (400 MHz) chemical shifts δ ($CDCl_3$):

[29-^{13}C]-(24*R*,28*S*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-28-ol, 21.952.

[29-^{13}C]-(24*R*,28*R*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-28-ol, 21.041.

[29-^{13}C]-(24*S*,28*R*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-28-ol, 22.198.

[29-^{13}C]-(24*S*,28*S*)-6β-Methoxy-3α,5-cyclo-5α-stigmastan-28-ol, 20.981. The isomers were pooled (39.4 mg, 82%) and used for the next step.

[29-^{13}C]-6β-Methoxy-3α,5-cyclo-5α-stigmast-24(28)-ene (11a,b). A solution of [29-^{13}C]-6β-methoxy-3α,5-cyclo-5α-stigmastan-28-ol (39.4 mg) in pyridine was treated with 0.5 mL of phosphorus oxychloride for 2 days at 25 °C. The mixture was poured into dilute hydrochloric acid and extracted with ether. The organic layer was dried and evaporated. Chromatography over silica gel (eluent hexanes/ether, 19:1) gave a 2:1 mixture of the *E* and *Z* isomers (32.9 mg, 87%). ^{13}C NMR (400 MHz) chemical shifts, δ ($CDCl_3$), were assigned by comparison with the natural abundance ^{13}C NMR of (*E*)-stigmasta-5,24(28)-dien-3β-ol (fucosterol, **11a-N**) and (*Z*)-stigmasta-5,24(28)-dien-3β-ol (isofucosterol, **11b-N**):

[29-^{13}C]-(*E*)-6β-Methoxy-3α,5-cyclo-5α-stigmast-24(28)-ene (11a-M), 13.341.

[29-^{13}C]-(*Z*)-6β-Methoxy-3α,5-cyclo-5α-stigmast-24(28)-ene (11b-M), 12.913.

[29-^{13}C]-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastanes (12a–d). The above mixture of [29-^{13}C]fucosterol (**11a-M**) and isofucosterol (**11b-M**) *i*-methyl ethers (32.9 mg) was treated as described above to give a mixture of the four isomeric C-29 ^{13}C-labeled dichlorocyclopropanes (**12a–d**) (36.1 mg, 92%), which were separated by reverse-phase HPLC (MeOH). ^{13}C NMR (400 MHz) δ ($CDCl_3$):

[29-^{13}C]-(24*R*,28*R*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12a-M), 10.115.

[29-^{13}C]-(24*S*,28*S*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12b-M), 10.314.

[29-^{13}C]-(24*R*,28*S*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12c-M), 9.487.

[29-^{13}C]-(24*S*,28*R*)-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-stigmastane (12d-M), 9.512.

[29-^{13}C]-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-stigmastanes (3a–d). The C-29 ^{13}C-labeled dichlorocyclopropanes (**12a–d**) were dechlorinated by the procedure described above. Thus 6.7 mg of **12a-M** was reduced to give 3.7 mg of **3a-M** and 0.7 mg of recovered starting material (71% based on recovered starting material). ^{13}C NMR (400 MHz) δ ($CDCl_3$):

[29-^{13}C]-(24*R*,28*S*)-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-stigmastane (3a-M), 14.552.

[29-^{13}C]-(24*S*,28*R*)-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-stigmastane (3b-M), 14.456.

[29-^{13}C]-(24*R*,28*R*)-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-stigmastane (3c-M), 13.668.

[29-^{13}C]-(24*S*,28*S*)-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-stigmastane (3d-M), 13.671.

[26,27,29,30-$^{13}C_4$]-28-Methylstigmastadien-3β-ols (7–9): Stigmasta-28-one *i*-methyl ether[17,12a] (48.2 mg) was treated with [^{13}C]CH_3MgI followed by $POCl_3$ dehydration[18] to give the *i*-methyl ethers **7-M**, **8a-M**, and **9b-M** bearing the carbon-13 label in the

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(17) Busca, G.; Nicotra, F.; Ronchetti, F.; Russo, G. *Gazz. Chim. Ital.* **1978**, *108*, 665–669.

(18) Ohtaka, H.; Morisaki, M.; Ikekawa, N. *J. Org. Chem.* **1973**, *38*, 1688–1691.

26- and 27-positions (34.6 mg, 72%). Hydrolysis of 9.5 mg of the mixture as described above followed by HPLC separation gave the following (^{13}C NMR (400 MHz), δ ($CDCl_3$)):

[26,27-$^{13}C_2$]-(24*R*)-28-Methylstigmasta-5,25-dien-3β-ol (9a-N) (25%), 112.353, 18.883.

[26,27-$^{13}C_2$]-(24*S*)-28-Methylstigmasta-5,25-dien-3β-ol (9b-N) (43%), 111.868, 18.924.

[26,27-$^{13}C_2$]-28-Methylstigmasta-5,23-dien-3β-ol (7-N) (32%), 20.796, 19.703.

Direct isomerization of the remaining 25.1 mg with 3 mL of 10% TFA/C_6H_6 (25 °C, 14 h) followed by hydrolysis of the trifluoroacetates with 5% KOH/MeOH (25 °C, 10 min) gave a mixture of [26,27,29,30-$^{13}C_4$]-28-methylstigmastadienols (**7–9**) (22.4 mg, 89%). Separation by HPLC gave the following (^{13}C NMR (400 MHz), δ ($CDCl_3$)):

[26,27,29,30-$^{13}C_4$]-(24*R*)-28-Methylstigmasta-5,25-dien-3β-ol (9a-N) (18%), 112.353, 21.618, 20.856, 18.883.

[26,27,29,30-$^{13}C_4$]-(24*S*)-28-Methylstigmasta-5,25-dien-3β-ol (9b-N) (14%), 111.868, 21.493, 20.849, 18.924.

[26,27,29,30-$^{13}C_4$]-28-Methylstigmasta-5,23-dien-3β-ol (7-N) (13%), 24.796, 24.788, 21.163, 20.950.

[26,27,29,30-$^{13}C_4$]-28-Methylstigmasta-5,24-dien-3β-ol (8-N) (55%), 21.405, 21.321, 20.796, 19.703.

Acid-Catalyzed Ring Opening of [29-^{13}C]-3c,d. The C-29 ^{13}C-labeled cyclopropanes were treated with 10% TFA/C_6H_6 at 25 °C for 1 h. The trifluoroacetate esters were hydrolyzed with 5% KOH/MeOH, and the products were extracted with ether and water. The sterols were purified by preparative TLC (hexanes/ether, 1:1) and **7-N** was isolated as described above by HPLC. ^{13}C NMR (400 MHz) δ ($CDCl_3$): **7** deriving from **3c** mainly (60%) 21.163; **7** deriving from **3d** mainly (60%) 20.950.

Synthesis of ^{13}C-Labeled 24,28-Methylene-5-ergosten-3β-ol (4): [28-^{13}C]-6β-Methoxy-3α,5-cyclo-5α-ergost-24(28)-ene. A solution of 300 mg of [^{13}C]methyltriphenylphosphonium iodide (99.9% ^{13}C) in THF was treated with *n*-BuLi (0.5 mL, 1.5 M hexane) at 0 °C for 45 min. A solution of 24-ketocholesterol *i*-methyl ether[19] in dry THF was added. After 14 h at 25 °C the reaction mixture was poured into water and extracted with ether. The organic layer was dried and evaporated. Purification by silica gel chromatography (eluent hexanes/ether, 19:1, followed by 9:1) gave 46.9 mg of starting material and 135.8 mg of [28-^{13}C]-24-methylenecholesterol (89% based on recovered starting material): ^{13}C NMR (400 MHz) ($CDCl_3$) δ 105.810.

[28-^{13}C]-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-ergostanes. [28-^{13}C]-24-Methylenecholesterol *i*-methyl ether (135.8 mg) was treated with dichlorocarbene as described above followed by silica gel chromatography (154.0 mg, 94%). The two isomers were separated by HPLC. The stereochemistry of these products was not assigned.

[28-^{13}C]-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-ergostane A: HPLC RT 88 min; ^1H NMR (400 MHz) ($CDCl_3$) δ 3.323 (3 H, s, OMe), 1.091 (3 H, d, *J* = 6.8 Hz), 1.003 (3 H, d, *J* = 7.0 Hz) C26 and C27, 1.018 (3 H, s, C19), 0.924 (3 H, d, *J* = 6.8 Hz, C21), 0.708 (3 H, s, C18); ^{13}C NMR (400 MHz) ($CDCl_3$) δ 31.326.

[28-^{13}C]-24,28-(Dichloromethylene)-6β-methoxy-3α,5-cyclo-5α-ergostane B: HPLT RT 90 min; ^1H NMR (400 MHz) ($CDCl_3$) δ 3.324 (3 H, s, OMe), 1.098 (3 H, d, *J* = 6.8 Hz), 1.006 (3 H, d, *J* = 7.0 Hz) C26 and C27, 1.019 (3 H, s, C19), 0.939 (3 H, d, *J* = 6.6 Hz, C21), 0.705 (3 H, s, C18); ^{13}C NMR (400 MHz) ($CDCl_3$) δ 30.995.

[28-^{13}C]-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-ergostanes (4a,b). The above C-28 ^{13}C-labeled dichlorocyclopropanes A (19.7 mg) and B (13.8 mg) were reduced as described above to yield:

[28-^{13}C]-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-ergostane (4a-M) (17.1 mg, 100%): ^{13}C NMR (400 MHz) ($CDCl_3$) δ 9.904.

[28-^{13}C]-24,28-Methylene-6β-methoxy-3α,5-cyclo-5α-ergostane (4b-M) (11.7 mg, 98%): ^{13}C NMR (400 MHz) ($CDCl_3$) δ 9.999.

24,28-Methylene-5-ergosten-3β-ol (4-N). 24-Methylenecholesterol *i*-methyl ether (70 mg) was treated according to the above sequence (33 mg, 90% overall) (mp 159–162 °C): ^1H NMR (400 MHz) ($CDCl_3$) δ 5.356 (1 H, m, C6), 1.006 (3 H, s, C19), 0.889 (3 H, d, *J* = 6.5 Hz), 0.865 (3 H, d, *J* = 6.8 Hz), 0.843 (3 H, d, *J* = 6.8 Hz) C21, C26 and C27, 0.702 (3 H, s, C18), 0.230 (2 H, m), 0.158 (2 H, m) C28 and C29; MS, *m/z* (relative intensity) 412 (M, 6), 397 (1), 327 (2), 314 (3), 300 (7), 299 (5), 271 (26), 213 (14), 55 (100).

Acid-Catalyzed Ring Opening of [28-^{13}C]-24,28-Methylene-6β-methoxy-3α,5-cycloergostan-3β-ols (4a,b). The diastereomerically labeled C-29 ^{13}C-labeled cyclopropanes were treated with 10% TFA/C_6H_6 at 25 °C 23 h. The trifluoroacetate esters were hydrolyzed with 5% KOH/MeOH, and the products were extracted with ether and water. Preparative TLC (hexanes/ether, 1:1) gave two more polar bands (approximately 25%) as well as the expected sterols. These polar side products were shown to be stigmast-5-ene-3β,24-diol[3] and stigmast-5-ene-3β,28-diol[20] by NMR and mass spectra. These are believed to arise from trifluoroacetate capture of the intermediate carbonium ions. This side reaction can be suppressed by the addition of a small amount of water to the reaction mixture. HPLC of the sterol band gave four fractions (^{13}C NMR (400 MHz) ($CDCl_3$) δ (C28 and C29 determined from APT spectra):

Fraction 1 (51 min) (17%) **[28,29-$^{13}C_2$]stigmasta-5,25-dien-3β-ol**, 25.552 (C28), 13.465 (C29).

Fraction 2 (54 min) (16%) **[28,29-$^{13}C_2$]stigmasta-5,23(*E*)-dien-3β-ol**, 24.441 (C28), 14.128 (C29).

Fraction 3 (56 min) (14%) a 10:29:1 mixture: **[28,29-$^{13}C_2$]-stigmasta-5,23(*Z*)-dien-3β-ol**, 23.296 (C28), 13.724 (C29); **[28,29-$^{13}C_2$]stigmasta-5,24(28)(*E*)-dien-3β-ol (8a-N)**, 115.452 (C28), 13.341 (C29); **[28,29-$^{13}C_2$]stigmasta-5,24(28)(*Z*)-dien-3β-ol (8b-N)**, 116.326 (C28), 12.913 (C29).

Fraction 4 (59 min) (53%) **[28,29-$^{13}C_2$]stigmasta-5,24-dien-3β-ol**, 25.445 (C28), 13.252 (C29). The ratio of the NMR integrals (C28/C29) of fraction 4 was 1.10 when derived from cyclopropane A, and 0.99 when derived from cyclopropane B.

Synthesis of *tert*-Butylcyclopropyl Sterol 5. 24-Methyl-6β-methoxy-3α,5-cyclo-26,27-dinorergostan-23-one (15-M). A solution of 250 mg of 6β-methoxy-3α,5-cyclo-24-norcholane-23-nitrile[5d] in 30 mL of dry ether was treated with 5 mL of 1.7 M *t*-BuLi/pentane under Ar for 15 min at 0 °C and 10 min at 25 °C. After hydrolysis with 15 mL of 10% HOAc for 20 min the mixture was poured into water and extracted with ether. The crude product was purified by silica gel chromatography (eluent hexanes/ether, 9:1) (241 mg, 83%): ^1H NMR (400 MHz) ($CDCl_3$) δ 3.313 (3 H, s, OMe), 2.359 (2 H, d, *J* = 6.7 Hz, C22), 1.106 (9 H, s, *t*-Bu), 1.012 (3 H, s, C19), 0.856 (3 H, d, *J* = 6.4 Hz, C21), 0.753 (3 H, s, C18).

23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-cholan-23-ols. A solution of 234.1 mg of the *tert*-butyl ketone (**15-M**) in 5 mL of dry ether was added to a solution of methylmagnesium iodide (from 150 μL of methyl iodide and 68 mg of magnesium) in 5 mL of ether. After 12 h at 25 °C the mixture was poured into water and extracted with ether. The crude product was purified by silica gel chromatography (eluent hexanes/ether, 9:1, followed by 4:1) to give two epimeric alcohols, which were pooled, 232.2 mg (95%). Stereochemistry was assigned by inspection of a model. The major isomer is considered to arise from attack on the least hindered face of the carbonyl group.

(23*S*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-cholan-23-ol (68%): ^1H NMR (400 MHz) ($CDCl_3$) δ 3.329 (3 H, s, OMe), 1.140 (3 H, s, C24), 1.102 (2 H, d, *J* = 8.1 Hz, C23), 1.084 (3 H, d, *J* = 6.4 Hz, C21), 1.023 (3 H, s, C19), 0.913 (9 H, s, *t*-Bu), 0.768 (3 H, s, C18).

(23*R*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-cholan-23-ol (32%): ^1H NMR (400 MHz) ($CDCl_3$) δ 3.322 (3 H, s, OMe), 1.131 (3 H, s, C24), 1.018 (3 H, d, *J* = 6.4 Hz, C21), 1.020 (3 H, s, C19), 0.915 (9 H, s, *t*-Bu), 0.768 (3 H, s, C18).

Dehydration of *tert*-Butyl Methyl 23-Alcohols. A solution of 231 mg of the alcohols in 4 mL of pyridine was treated with 0.3 mL of $POCl_3$. After 48 h at 25 °C the mixture was poured into dilute hydrochloric acid and extracted with ether. The crude product was purified by silica gel chromatography (eluent hexanes/ether, 39:1) (205 mg, 93%). Separation of 271 mg of the

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mixture by 15% $AgNO_3$/silica gel chromatography (eluent hexanes/ether, 39:1) gave two isomeric olefins:

(*E*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-chol-22-ene (16-M) (181.5 mg, 67%): ^1H NMR (400 MHz) ($CDCl_3$) δ 4.956 (1 H, d, J = 9.5 Hz, C22), 3.324 (3 H, s, OMe), 1.598 (3 H, s, C24), 1.026 (3 H, s, C19), 0.998 (9 H, s, *t*-Bu), 0.914 (3 H, d, J = 6.6 Hz, C21), 0.747 (3 H, s, C18); MS, m/z (relative intensity) 412 (M, 9), 397 (6), 357 (11), 314 (12), 282 (10), 255 (18), 253 (18), 125 (100), 69 (95).

23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-chol-23-ene (14-M) (89.8 mg, 33%): ^1H NMR (400 MHz) ($CDCl_3$) δ 4.917 (1 H, s, C24), 4.669 (1 H, s, C24), 3.328 (3 H, s, OMe), 1.039 (9 H, s, *t*-Bu), 1.026 (3 H, s, C19), 0.880 (3 H, d, J = 6.1 Hz, C21), 0.760 (3 H, s, C18); MS, m/z (relative intensity) 412 (M, 4), 397 (7), 357 (16), 314 (31), 282 (27), 255 (10), 253 (17), 55 (100).

23,24-(Dichloromethylene)-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholanes (17a,b). Dichlorocyclopropanation of the *tert*-butyl olefin **14-M** (89.8 mg) as described above gave a 1:9 mixture of the two diastereomers **17a-M** and **17b-M** (65.9 mg, 61%). Stereochemistry was assigned by inspection of a model. The major isomer is considered to arise from attack on the least hindered face of the double bond. The isomers were separated by HPLC.

(23*R*)-23,24-(Dichloromethylene)-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (17a-M): HPLC RT 80 min (10%); ^1H NMR (400 MHz) ($CDCl_3$) δ 3.328 (3 H, s, OMe), 1.151 (9 H, s, *t*-Bu), 1.016 (3 H, s, C19), 0.946 (3 H, d, J = 6.6 Hz, C21), 0.722 (3 H, s, C18).

(23*S*)-23,24-(Dichloromethylene)-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (17b-M): HPLC RT 84 min (90%); ^1H NMR (400 MHz) ($CDCl_3$) δ 3.319 (3 H, s, OMe), 1.110 (9 H, s, *t*-Bu), 1.016 (3 H, s, C19), 0.704 (3 H, s, C18).

23,24-Methylene-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (5-M): Birch reduction of 30.1 mg of *tert*-butyldichlorocyclopropane (**17-M**) as described above gave the *tert*-butylcyclopropane (21.8 mg, 84%): ^1H NMR (400 MHz) ($CDCl_3$) δ 3.322 (3 H, s, OMe), 1.016 (3 H, s, C19), 0.904 (3 H, d, J = 5.5 Hz, C21), 0.823 (9 H, s, *t*-Bu), 0.702 (3 H, s, C18), 0.498 (2 H, m, cyclopropane), 0.094 (2 H, m, cyclopropane).

^{13}C-Labeled *tert*-Butylcyclopropanes (5a,b). The above sequence of reactions was used. The ^{13}C label was introduced by reaction of [^{13}C]MeMgI with the *tert*-butyl ketone **15-M** to give the following (^{13}C NMR (400 MHz) ($CDCl_3$) δ):

[24-^{13}C]-(23*S*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-cholan-23-ol, 21.402.

[24-^{13}C]-(23*R*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-cholan-23-ol, 21.163.

[24-^{13}C]-(*E*)-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-chol-22-ene (16-M), 13.000.

[24-^{13}C]-23-*tert*-Butyl-6β-methoxy-3α,5-cyclo-5α-chol-23-ene (14-M), 106.712.

[24-^{13}C]-(23*R*)-23,24-(Dichloromethylene)-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (17a-M), 28.291.

[24-^{13}C]-(23*S*)-23,24-(Dichloromethylene)-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (17b-M), 26.568 (very broad).

Birch reduction of ^{13}C-labeled dichlorocyclopropanes **22a** and **22b** gave C-24 ^{13}C-labeled cyclopropanes **5a** and **5b**, respectively:

[24-^{13}C]-(23*R*)-23,24-Methylene-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (5a-M), 8.083.

[24-^{13}C]-(23*S*)-23,24-Methylene-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (5b-M), 7.564.

Acid-Catalyzed Ring Opening of ^{13}C-Labeled *tert*-Butylcyclopropanes (5a,b). Diastereomerically labeled C-24 ^{13}C-labeled cyclopropanes **5a** and **5b** were treated with 10% TFA/C_6H_6 at 25 °C for 15 h. HPLC of the products of each diastereomer showed the same mixture for both (^{13}C NMR assignments based on APT spectra, ^1H NMR and mass spectra illustrated for the unlabeled compounds).

[24,25-$^{13}C_2$]-23-*tert*-Butyl-26,27-dinorcholesta-5,22(*E*)-dien-3β-ol (18-N) (31:): HPLC RT 54 min; mp 158–159 °C; ^{13}C NMR (400 MHz) ($CDCl_3$) δ 20.890 (C24), 15.818 (C25); ^1H NMR (400 MHz) ($CDCl_3$) δ 5.351 (1 H, m, C6), 4.941 (1 H, d, J = 10.1 Hz, C22), 1.015 (3 H, s, C19), 1.004 (9 H, s, *t*-Bu), 0.990 (3 H, t, J = 7.1 Hz, C25), 0.965 (3 H, d, J = 6.3 Hz, C21), 0.718 (3 H, s, C18); MS, m/z (relative intensity) 412 (M, 6), 356 (2), 355 (4), 354 (3), 300 (20), 271 (31), 255 (13), 139 (32), 83 (100), 69 (79). The ratio of the ^{13}C NMR integrals (C24/C25) was 0.93 when derived from cyclopropane **5a** and 1.36 when derived from cyclopropane **5b**.

[25,26-$^{13}C_2$]-23,24-Dimethyl-27-norergosta-5,22(*E*)-dien-3β-ol (19-N) (39%): HPLC RT 58 min; mp 167–168 °C; ^{13}C NMR (400 MHz) ($CDCl_3$) δ 33.116 (C25), 9.021 (C26); ^1H NMR (400 MHz) ($CDCl_3$) δ 5.351 (1 H, m, C6), 4.906 (1H, d, J = 9.5, C22), 1.531 (3 H, s, C28), 1.015 (3 H, s, C19), 0.717 (3 H, s, C18), 0.653 (3 H, t, J = 7.4, C26); MS, m/z (relative intensity) 412 (M, 9), 342 (7), 340 (4), 300 (19), 271 (35), 255 (12), 139 (32), 111 (56), 83 (86), 69 (100). The ratio of the ^{13}C NMR integrals (C28/C29) was 1.12 when derived from cyclopropane **5a** and 1.09 when derived from cyclopropane **5b**.

[24,25-$^{13}C_2$]-23-*tert*-Butyl-26,27-dinorcholesta-5,23(*E*)-dien-3β-ol (20-N) (8%): HPLC RT 64 min; mp 132–135 °C; ^{13}C NMR (400 MHz) ($CDCl_3$) δ 118.422 (C24), 14.884 (C25); ^1H NMR (400 MHz) ($CDCl_3$) δ 5.506 (1 H, q, J = 6.7 Hz, C24), 5.360 (1 H, m, C6), 1.569 (3 H, d, J = 6.7 Hz, C25), 1.043 (9 H, s, *t*-Bu), 1.012 (3 H, s, C19), 0.836 (3 H, d, J = 6.6 Hz, C21), 0.694 (3 H, s, C18); MS, m/z (relative intensity) 412 (M, 0.5), 300 (100), 285 (18), 283 (26), 282 (15), 267 (15), 241 (9), 215 (13), 133 (36), 69 (70), 57 (59), 55 (85).

[24-^{13}C]-23,24-Methylene-23-*tert*-butyl-5-cholen-3β-ol (5-N) (22%): HPLC RT 70 min; mp 185–186 °C; ^1H NMR (400 MHz) ($CDCl_3$) δ 5.356 (1 H, m, C6), 1.002 (3 H, s, C19), 0.911 (3 H, d, J = 5.2 Hz, C21), 0.821 (9 H, s, *t*-Bu), 0.667 (3 H, s, C18), 0.498 (2 H, m, cyclopropane), 0.094 (2 H, m, cyclopropane); MS, m/z (relative intensity) 412 (M, 3), 356 (6), 314 (5), 300 (25), 283 (13), 271 (15), 57 (100), 55 (62).

22,22-Dideuterio-23,24-methylene-23-*tert*-butyl-6β-methoxy-3α,5-cyclo-5α-cholane (5-M). The same sequence was used as described above. Deuterium was introduced by heating 84 mg of *tert*-butyl ketone (**15-M**) under reflux with a mixture of 20 mL of CH_3OD (99%), 2 mL of D_2O (99.9%), and 0.2 g of KO*t*-Bu for 48 h. A deuterium isotope effect in the $POCl_3$ dehydration led to an improved yield of the desired intermediate (**14-M**) (52% of olefin mixture): MS, m/z (relative intensity) 414 (M, 4), 358 (4), 300 (26), 283 (13), 271 (17), 57 (100), 55 (41).

Alternate Synthesis of 18 and 20. The same sequence was used as described above with the substitution of ethylmagnesium iodide for methylmagnesium iodide; **18** and **20** were obtained in a ratio of 1:2. All spectral properties were identical with those of the samples obtained from cyclopropane ring opening.

Alternate Synthesis of 19. The same sequence was used as described above with the substitution of the *tert*-amyl ketone for the *tert*-butyl ketone (**15-M**). Attempts to react *tert*-amylmagnesium chloride with 6β-methoxy-3α,5-cyclo-24-norcholane-23-nitrile[5d] or 6β-methoxy-3α,5-cyclocholan-23-one failed. The *tert*-amyl ketone was finally obtained by reaction of the above Grignard reagent with 6β-methoxy-3α,5-cyclo-24-norcholan-23-al and oxidation of the resulting alcohol with pyridinium chlorochromate. Compound **19-M** was formed together with **21-M** from the dehydration in a 3:1 ratio. All spectral properties of **19** were identical with those of the product obtained from cyclopropane ring opening.

23-Methylene-24-methyl-27-norergost-5-en-3β-ol (21-N): HPLC RT 68 min; ^1H NMR (400 MHz) ($CDCl_3$) δ 5.356 (1 H, m, C6), 4.873 (1 H, s, 23-methylene), 4.760 (1 H, s, 23-methylene), 1.345 (2 H, q, J = 7.9 Hz, C25), 1.014 (3 H, s, C19), 0.992 (6 H, s, C24 methyls), 0.903 (3 H, d, J = 6.4 Hz, C21), 0.726 (1 H, s, C18), 0.710 (3 H, t, J = 7.5 Hz, C26); MS, m/z (relative intensity) 412 (M, 2), 300 (100), 285 (31), 283 (22), 282 (24), 271 (24), 267 (32), 241 (19), 215 (20), 213 (17).

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