Structural and Stereochemical Applications of Mass Spectrometry in the Marine Sterol Field.[1] Synthesis and Electron Impact Induced Mass Spectral Fragmentation of Δ^{24}- and $\Delta^{24(28)}$-3β-Hydroxy-Δ^5-sterols[2]

Ian J. Massey and Carl Djerassi*

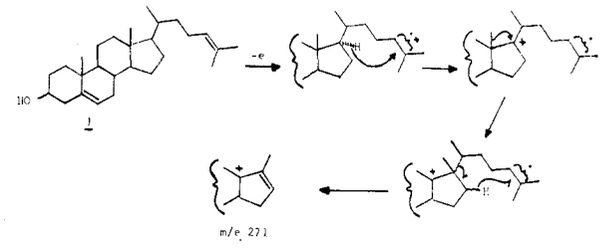
Department of Chemistry, Stanford University, Stanford, California 94305

Received January 9, 1979

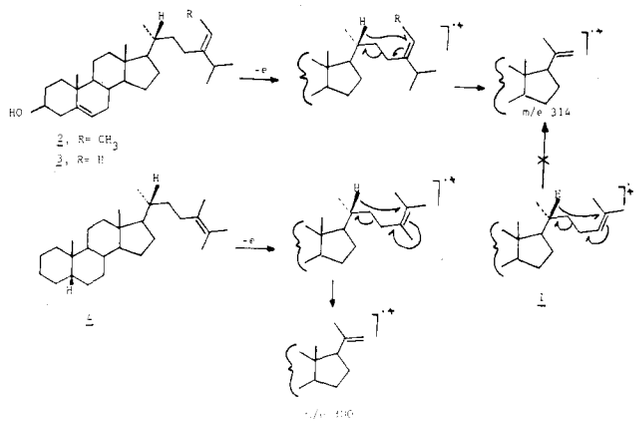
3β-Hydroxy-Δ^5-sterols possessing a C-24 or C-24(28) unsaturated side chain undergo two competing mass spectral fragmentation processes corresponding to (a) cleavage of the 22–23 bond with the transfer of one hydrogen to the departing neutral fragment and (b) loss of the C-17 substituent with the transfer of two hydrogen atoms from the steroid nucleus. The use of compounds labeled with deuterium at C-20 has allowed us to demonstrate that process (a) occurs by a McLafferty rearrangement involving the C-20 proton. The relative importance of these two fragmentation processes is found to be dependent upon the substitution type of the side chain unsaturation and upon the stereochemistry at C-20. Attention is drawn to the utility of such mass spectral fragmentations in structure elucidations of new marine sterols. Synthetic routes to the labeled compounds started from the readily available stigmasterol and were designed to be used also for the synthesis of ^{13}C- and ^{3}H-labeled desmosterol, fucosterol, and 24-methylenecholesterol—three prime candidates for biochemical examination of marine sterol side chain biosynthesis.

The past few years has seen an increasing interest in sterols of marine origin, which has led to the discovery of a large number of novel compounds.[3] The recent use of a variety of separation techniques has demonstrated the complexity of the sterol components of marine extracts—up to 50 sterols from one organism.[4] However, the quantity of each sterol in these mixtures is frequently so small as to preclude the isolation and structure determination by the traditional techniques. Mass spectrometry in conjunction with gas chromatography (GC–MS) has become a vital tool in the examination of sterol extracts, and, in view of the quantities available, structures must frequently be assigned on the basis of a single GC–MS.[5] Clearly therefore, an understanding of those fragmentation processes which are characteristic of certain functional groups in the steroid framework is essential for investigations of this type. The most interesting feature of marine sterols is the side chain, which is usually unsaturated or has a cyclopropane ring, which mass spectrometrically bears considerable resemblance to an olefin.[6] Those fragmentations which are associated with these centers of unsaturation are therefore of particular importance to these investigations, but with the exception of one labeling study,[7] all conclusions about such fragmentations are based on circumstantial evidence.

Scheme I



Scheme II



We have observed that the mass spectral fragmentation processes associated with C-24 unsaturated 3β-hydroxy-Δ^5-sterols are dependent on the substitution type of the side chain unsaturation. This point is well illustrated by a comparison of the spectra[8] of desmosterol (1) and fucosterol (2). Thus the base peak in the spectrum of desmosterol is at m/e 271, a fragment which corresponds to loss of the side chain together with two hydrogens from the steroid nucleus. Labeling experiments[7] with a relevant model sterene revealed that one hydrogen comes exclusively from C-17 and that 71% of the second comes from C-16, C-14, and C-12. The proposed mechanism for the formation of this ion is shown in Scheme I for the transfer of the C-16 and C-17 hydrogens. Similar mechanisms can be written to include the C-14 and C-12 hydrogens each

(1) Paper CCLII in the series "Mass Spectrometry in Structural and Stereochemical Problems". For the previous paper see K. K. Mayer, I. Matolovic, E. Fischer, and C. Djerassi, *Tetrahedron Lett.*, in press.

(2) Financial support by the National Institutes of Health (Grant Nos. AM-04257 and RR-00612) is gratefully acknowledged.

(3) For leading references see L. J. Goad in "Biochemical and Biophysical Perspectives in Marine Biology", D. C. Malins and J. R. Sargent, Eds., Academic Press, New York, 1976, pp 213-318; F. J. Schmitz in "Marine Natural Products", P. J. Scheuer, Ed., Academic Press, New York, 1978, Chapter 5; L. Minale and G. Sodano in "Marine Natural Products Chemistry", D. J. Faulkner and W. H. Fenical, Eds., Plenum Press, New York, 1977, pp 87–109.

(4) S. Popov, R. M. K. Carlson, A. Wegmann, and C. Djerassi, *Steroids*, **28**, 699 (1976); J. A. Ballantine and K. Williams, *Tetrahedron Lett.*, 1547 (1977); M. Kobayshi and M. Mitsuhashi, *Steroids*, **26**, 605 (1975).

(5) J. A. Ballantine, J. C. Roberts, and R. J. Morris, *Biomed. Mass Spectrom.*, **3**, 14 (1976); C. Francisco, G. Combaut, J. Teste, and B. F. Maume, *Biochim. Biophys. Acta*, 487 (1977); A. M. K. Wardroper, J. R. Maxwell, and R. J. Morris, *Steroids*, **32**, 203 (1978); S. Popov, R. M. K. Carlson, A. M. Wegmann, and C. Djerassi, *Tetrahedron Lett.*, 3491 (1976).

(6) J. R. Dias and C. Djerassi, *Org. Mass Spectrom.*, **7**, 753 (1973); B. N. Ravi, W. C. M. C. Kokke, C. Delseth, and C. Djerassi, *Tetrahedron Lett.*, in press.

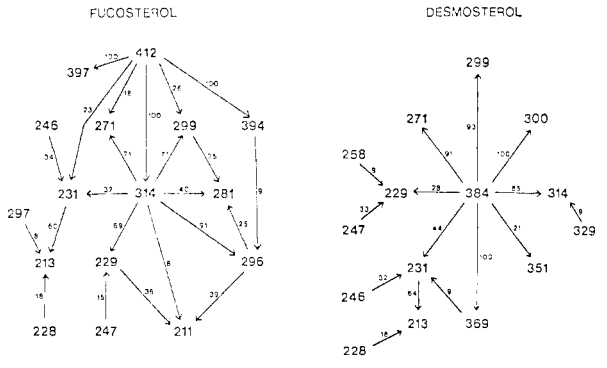
(7) S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).

(8) A. Wegmann, *Anal. Chem.*, **50**, 830 (1978).

0022-3263/79/1944-2448$01.00/0

Scheme III

METASTABLE DEFOCUSSING



with the formation of allylic carbonium ions. This type of fragmentation also gives rise to prominent fragments in the spectra of C-22 and C-23 unsaturated sterols[7] and is therefore common to a variety of unsaturated side chain sterols.

The mass spectrum of fucosterol (**2**) also has a fragment at m/e 271, but this is of low intensity (13%) compared to the intensity of its base peak at m/e 314. This m/e 314 fragment, which is characteristic of $\Delta^{24(28)}$-unsaturated sterols has been suggested[9] to arise by a McLafferty fragmentation involving the C-20 hydrogen (Scheme II) although no labeling evidence has been presented to substantiate this suggestion. While a similar McLafferty rearrangement would appear equally feasible for desmosterol (**1**) (Scheme II), the m/e 314 fragment is virtually absent (2%) in this case. However, the base peak in the spectra of 24-methylenecholesterol (**3**) and of 24-methyl-5β-cholest-24-ene (**4**)[7] once again appears to arise by such a McLafferty rearrangement.

The different fragmentations initiated by the unsaturated side chains of desmosterol (**1**) on the one hand and of fucosterol (**2**), 24-methylenecholesterol (**3**), and the sterene **4** on the other could easily be explained if the desmosterol actually does undergo a McLafferty rearrangement to produce a mass 314 ion which is in a higher energy state than that produced from **2**, **3**, or **4**, with the result that it undergoes rapid further fragmentation to m/e 271, a possibility supported by the observation that the mass spectrum of 20-methylenepregn-5-en-3β-ol shows a fragment ion of mass 271.[10] In order to eliminate this possibility, we recorded the low electron voltage (12 eV) spectra of desmosterol (**1**) and of fucosterol (**2**), and a complete high-resolution and metastable defocusing study was performed on these two sterols.

The low electron voltage study showed that for desmosterol the base peak remained at m/e 271 with no increase in the relative intensity of the m/e 314 fragment as compared to the case for the 70-eV spectrum. In the case of fucosterol, the m/e 314 fragment remained the base peak and the m/e 271 fragment showed a decrease in relative intensity.

The metastable defocusing data (Scheme III) show that in the case of desmosterol (**1**), the molecular ion (m/e 384) is the major precursor of m/e 271 and of most of the other fragments above m/e 229. However, the situation is markedly different for fucosterol (**2**) in which the m/e 314 fragment, itself derived directly from the molecular ion (m/e 412), is the major progenitor of the lower mass fragments including m/e 271. These results indicate that the m/e 271 fragment in the spectrum of fucosterol, which corresponds to the formal loss of the side chain plus two hydrogens, must arise by a mechanism predominantly different from that established[7] for formation of the "same" ion in the case of desmosterol (**1**).

In view of these facts and the lack of any obvious explanation of the profound differences observed, it became necessary to (a) confirm the operation of a McLafferty rearrangement in the formation of the m/e 314 ion in the spectra of certain C-24 unsaturated side chain sterols and (b) investigate the effect of alkyl substitution of the side chain unsaturation on the fragmentation processes initiated by this unsaturation. In order to investigate the McLafferty fragmentation, we prepared the C-20-*d* labeled sterols **23**, **34**, **36**, and **46**. If formation of the m/e 314 fragment does involve a McLafferty rearrangement, then it is reasonable to expect that such a rearrangement might be influenced by the stereochemistry at C-20. If this is the case, mass spectrometry, in certain circumstances, might provide a sensitive probe to the stereochemistry at C-20, which in view of the recent isolation[11] of a number of C-20 iso sterols has obvious applications. These considerations led us to prepare the labeled C-20 iso sterols **24**, **35**, **37**, and **47**. To examine the influence of alkyl substitution on the side chain unsaturation, we also synthesized the sterols **49**, **55**, **57**, and **61**.

Synthesis

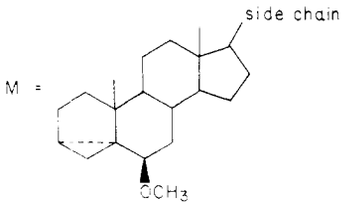
The syntheses used were selected on the basis of their possible extension to the synthesis of ^{13}C and ^{3}H side chain labeled desmosterol, fucosterol, and 24-methylenecholesterol for future biosynthetic investigations.

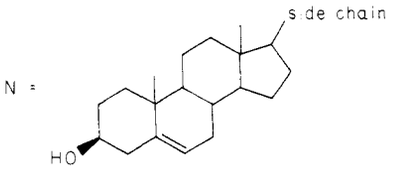
Stigmasterol (**5**) was the starting material for the synthesis of the deuterium-labeled compounds (see Chart I). Conversion of **5** into the 6β-methoxy-3α,5α-cyclo derivative **6**[12] followed by ozonolysis gave the aldehyde **7**[13] in which the C-20 proton is activated for hydrogen/deuterium exchange. Treatment of **7** with sodium in D_2O/CH_3OD under a nitrogen atmosphere[14] followed by reduction with $LiAlH_4$ gave a mixture of the 20*R* and 20*S* alcohols **10** and **11** which were separated by chromatography.[14] The less polar 20*R* alcohol **10** (55%) which has the unnatural C-20 configuration was, by mass spectrometry, a 96% isotopically pure monodeuterium compound. The NMR [δ 0.93 (3 H, s, C(21))] and mass spectra showed that the deuterium was located exclusively at C-20. The more polar alcohol **11** has the natural 20*S* configuration and was found to be 96% isotopically pure d_1. These two alcohols were the precursors for all of the required C-20 labeled compounds.

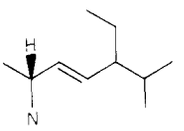
Labeled desmosterol (**23**) was prepared from the alcohol **11** by oxidation to the aldehyde **8** followed by a Wittig reaction to afford the (*E*)-Δ^{22}-α,β-unsaturated ester **12**.[14] Hydrogenation of **12** gave the saturated ester **14**, which was reduced with lithium aluminum hydride to afford the alcohol **16**. Oxidation of **16** to the aldehyde **19** followed by reaction with isopropylidenetriphenylphosphorane gave the *i*-methyl ether **21** which upon aqueous acid treatment afforded the labeled desmosterol (**23**). This sequence of reactions did not result in any impairment of either the isotopic or stereochemical integrity at C-20. The labeled 20-iso compound **24** was prepared in a similar manner from the alcohol **10**.

(9) J. Bergman, B. O. Lindgren, and C. M. Svahn, *Acta Chem. Scand.*, 19, 1661 (1965).
(10) I. J. Massey and C. Djerassi, to be submitted for publication.
(11) D. J. Vanderah and C. Djerassi, *Tetrahedron Lett.*, 683 (1977).
(12) J. A. Steele and E. Mosettig, *J. Org. Chem.*, **28**, 571 (1963).
(13) W. G. Salmond and M. C. Sobala, *Tetrahedron Lett.*, 1695 (1977).
(14) D. J. Vanderah and C. Djerassi, *J. Org. Chem.*, **43**, 1442 (1978).

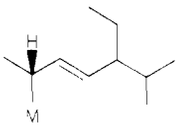
Chart I



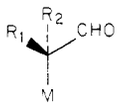




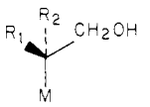
5



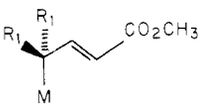
6



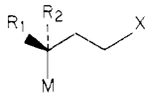
7, $R_1 = H$, $R_2 = CH_3$
8, $R_1 = D$, $R_2 = CH_3$
9, $R_1 = CH_3$, $R_2 = D$



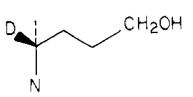
10, $R_1 = CH_3$, $R_2 = D$
11, $R_1 = D$, $R_2 = CH_3$



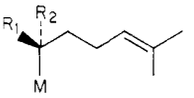
12, $R_1 = D$, $R_2 = CH_3$
13, $R_1 = CH_3$, $R_2 = D$



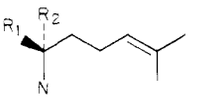
14, $R_1 = D$, $R_2 = CH_3$, $X = CO_2CH_3$
15, $R_1 = CH_3$, $R_2 = D$, $X = CO_2CH_3$
16, $R_1 = D$, $R_2 = CH_3$, $X = CH_2OH$
17, $R_1 = CH_3$, $R_2 = D$, $X = CH_2OH$
19, $R_1 = D$, $R_2 = CH_3$, $X = CHO$
20, $R_1 = CH_3$, $R_2 = D$, $X = CHO$



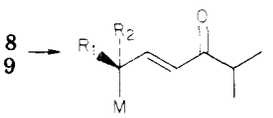
18



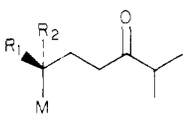
21, $R_1 = D$, $R_2 = CH_3$
22, $R_1 = CH_3$, $R_2 = D$



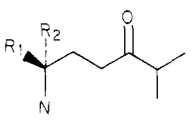
23, $R_1 = D$, $R_2 = CH_3$
24, $R_1 = CH_3$, $R_2 = D$



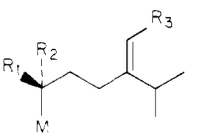
25, $R_1 = D$, $R_2 = CH_3$
26, $R_1 = CH_3$, $R_2 = D$



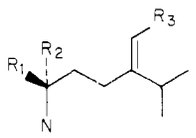
27, $R_1 = D$, $R_2 = CH_3$
28, $R_1 = CH_3$, $R_2 = D$



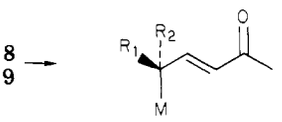
29, $R_1 = CH_3$, $R_2 = D$



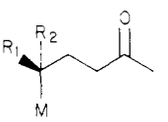
30, $R_1 = D$, $R_2 = CH_3$, $R_3 = H$
31, $R_1 = CH_3$, $R_2 = D$, $R_3 = H$
32, $R_1 = D$, $R_2 = CH_3$, $R_3 = CH_3$
33, $R_1 = CH_3$, $R_2 = D$, $R_3 = CH_3$



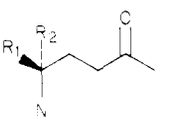
34, $R_1 = D$, $R_2 = CH_3$, $R_3 = H$
35, $R_1 = CH_3$, $R_2 = D$, $R_3 = H$
36, $R_1 = D$, $R_2 = CH_3$, $R_3 = CH_3$
37, $R_1 = CH_3$, $R_2 = D$, $R_3 = CH_3$



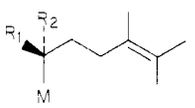
38, $R_1 = D$, $R_2 = CH_3$
39, $R_1 = CH_3$, $R_2 = D$



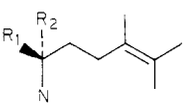
40, $R_1 = D$, $R_2 = CH_3$
41, $R_1 = CH_3$, $R_2 = D$



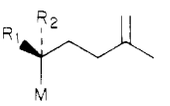
42, $R_1 = D$, $R_2 = CH_3$
43, $R_1 = CH_3$, $R_2 = D$



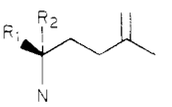
44, $R_1 = D$, $R_2 = CH_3$
45, $R_1 = CH_3$, $R_2 = D$



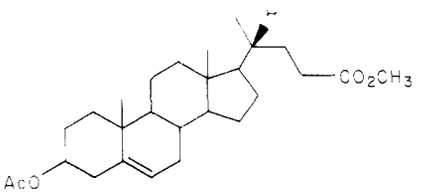
46, $R_1 = D$, $R_2 = CH_3$
47, $R_1 = CH_3$, $R_2 = D$



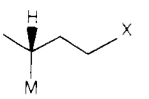
48, $R_1 = CH_3$, $R_2 = D$



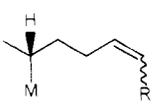
49, $R_1 = CH_3$, $R_2 = D$



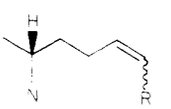
50



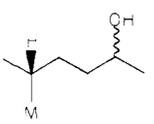
51, $X = CO_2CH_3$
52, $X = CH_2OH$
53, $X = CHO$



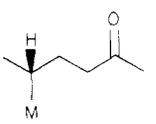
54, $R = H$
56, $R = CH_3$



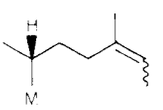
55, $R = H$
57, $R = CH_3$



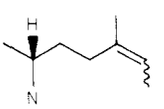
58



59



60



61

Labeled 24-methylenecholesterol (**34**) and its C-20 epimer **35** were prepared from the aldehydes **8** and **9**, respectively, by treatment with 2-butanone–methylenetriphenylphosphorane to afford the (*E*)-Δ^{22}-α,β-unsaturated ketones **25** and **26**. Hydrogenation gave the 24-keto compounds **27** and **28**, which upon treatment with methylenetriphenylphosphorane followed by acidic hydrolysis yielded the desired 24-methylenecholesterols **34** and **35**.

Table I. Relative Intensities of Selected Mass Spectral Fragments

compd	m/e M+ (intensity)	m/e 314, %	m/e 299, %	m/e 271, %
1	384 (29)	1	16	100
23	385 (22)	1	12	100
24	385 (7)	1	5	100
3	398 (20)	100	23	30
34	399 (15)	100	23	64
35	399 (9)	45	19	100
46[a]	399 (14)	69	24	35
47	399 (15)	30	11	100
2	412 (8)	100	21	14
36	413 (7)	100	19	13
37	413 (10)	100	20	50
49	371 (10)	22	10	100
55	356 (100)	1	8	52
61	384 (15)	100	32	23
57	370 (20)	2	12	100

[a] At 70 eV the base peak is at m/e 83.

The labeled (24Z)-stigmasta-5,24(28)-dien-3β-ols **36** and **37** were prepared from the 24-keto compounds **27** and **28** by treatment with ethylidenetriphenylphosphorane[15] followed by acidic hydrolysis. Since it has been demonstrated[16] that fucosterol (**2**) and its 24(28) isomer cannot be distinguished by mass spectrometry, **36** and **37** are good substitutes for the somewhat more difficultly obtained (*E*)-24(28) isomers.

Labeled 24-methylcholesta-5,24-dien-3β-ol (**46**) was prepared by treatment of the aldehyde **8** with acetylmethylenetriphenylphosphorane to afford the (*E*)-Δ^{22}-unsaturated ketone **38**. Hydrogenation gave the ketone **40** which upon treatment with isopropylidenetriphenylphosphorane and acidic hydrolysis yielded the labeled sterol **46**. The C-20 iso compound **47** was prepared by a similar reaction sequence on the aldehyde **9**.

The sterol **49** was generated from ketone **41** by treatment with methylenetriphenylphosphorane followed by acidic hydrolysis.

The unlabeled sterols **55**, **57**, and **61** needed to delineate the structural scope of the McLafferty rearrangement were synthesized from methyl 3β-acetoxycholenate (**50**). Conversion of **50** into the *i*-methyl ether **51** and reduction with $LiAlH_4$ gave the alcohol **52** which was oxidized with Collins' reagent[17] to the aldehyde **53**. Treatment with the appropriate Wittig reagents gave the *i*-methyl ethers **54** and **56** which upon acidic hydrolysis afforded the sterols **55** and **57**.

The sterol **61** was synthesized from the aldehyde **53** by treatment with methyllithium to afford the alcohol **58** followed by oxidation to the ketone **59**. Treatment with ethylidenetriphenylphosphorane and acidic hydrolysis gave **61** as a mixture of *E* and *Z* isomers.

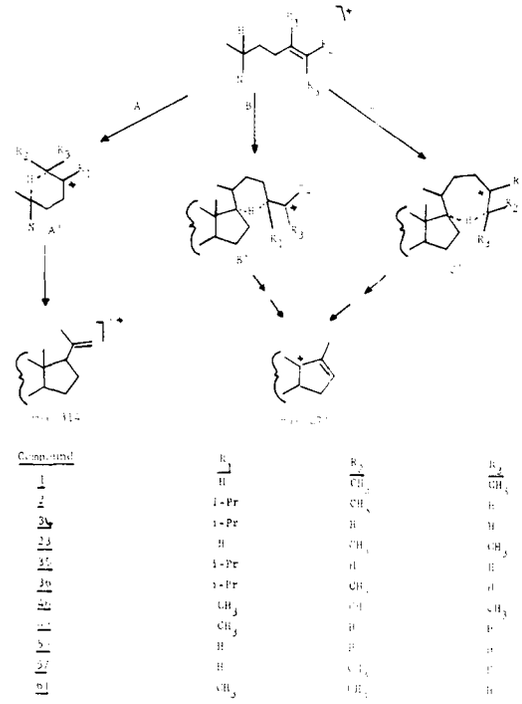
Discussion of Mass Spectral Fragmentations

The most important fragments (for the present discussion) in the spectra of the synthetic compounds and of the naturally occurring compounds **1**–**3** are shown in Table I. The data show a number of interesting features which can be summarized as follows: (a) Those compounds which in their unlabeled forms show, or are expected to show, a mass 314 ion in their spectra do not show any shift of this ion in the C-20-*d* labeled compounds thus indicating that the formation of this ion involves the loss of the C-20 hydrogen. (b) The m/e 314 fragment is important only in those compounds trisubstituted at C-24, and, in the absence of this substitution type, m/e 271 is the predominant fragment. (c) All compounds, which show a significant m/e 314 fragment, also show a m/e 271 fragment and the relative intensities of these two ions are dependent on the substitution of the side chain unsaturation (e.g., **34** vs. **49**) and, for a given substitution type, also on the stereochemistry at C-20 (e.g., **34** vs. **35**).

Table II. Summary of Metastable Defocusing Studies

compd	m/e 314, %	parents of m/e 314 (%)	m/e 271, %	parents of m/e 271 (%)
34	100	M+ (100)	64	M+ (56) m/e 314 (44)
35	44	M+ (100)	100	M+ (80) m/e 314 (20)
46	69	M+ (100)	35	M+ (65) m/e 314 (35)
2	100	M+ (100)	14	M+ (71) m/e 314 (18)
1	1	M+ (85)	100	M+ (91)

Scheme IV



Metastable defocusing studies were performed on (20*R*)-24-methylenecholesterol-20-*d* (**34**), (20*S*)-24-methylenecholesterol-20-*d* (**35**), and (20*R*)-24-methylcholesta-5,24-dien-3β-ol-20-*d* (**46**) and the results are shown in Table II together with a summary of those for desmosterol (**1**) and fucosterol (**2**). The data indicate that in these cases the mass 314 ion is formed directly from the molecular ion. In those instances where m/e 314 and m/e 271 are simultaneously present both the molecular ion and the mass 314 fragment are precursors of the mass 271 fragment and the importance of each precursor varies from compound to compound. When only m/e 271 is present, it is derived solely from the molecular ion.

The results obtained from the labeled compounds provide strong evidence for the formation of the mass 314 ion by a McLafferty rearrangement involving the C-20 proton. The transition state for the formation of the mass 314 ion can therefore be represented in a simplified manner as shown in Scheme IV (path A). The initial hydrogen

(15) J. P. Dusza, *J. Org. Chem.*, **25**, 93 (1960); W. Sucrow and B. Radüchel, *Chem. Ber.*, **102**, 2629 (1969).
(16) C. J. W. Brooks, B. A. Knights, W. Sucrow, and B. Radiichel, *Steroids*, **20**, 488 (1972).
(17) R. Ratcliff and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).

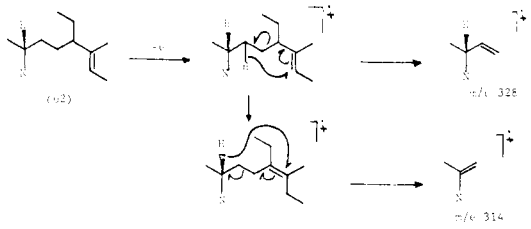
transfer for the multistep fragmentation to the presumably more stable (tertiary allylic) mass 271 ion can occur by either a six (path B) or seven (path C) membered transition state depending on the nature of R_1, R_2, and R_3. Clearly for **34**, **36**, **49**, and **61** transition state A′ is favored over B′ and C′ as the result of both the stabilizing effect of the C-24 alkyl substitutent and the ring size of the transition state; i.e., pathway A leads to a tertiary/tertiary radical cation via a six-membered cyclic transition state whereas pathway C leads to a tertiary/tertiary radical cation via the higher energy seven-membered transition state. The McLafferty fragmentation is therefore favored in these cases. For desmosterol (**1**), however, pathway B is preferred since this leads to the more stable tertiary/tertiary radical-cation intermediate. Formation of the mass 271 fragment is therefore favored in this case apparently to the extent of excluding the McLafferty fragmentation.

It appears that in some cases there is a rather delicate balance between the three possible pathways which is influenced not only by the nature of R_1 (i.e., secondary vs. tertiary ion) but also by that of R_2 and R_3. Thus the R_1 group (isopropyl) of **34** is able to stabilize A′ more effectively than R_1 (methyl) of **49** with the result that pathway C is able to compete more effectively with A in the case of **49** than in **34** thus accounting in part for the increased relative abundance of the mass 271 ion in the spectrum of **49** as shown in Table II. The effect of R_2 can be seen by a comparison of the spectra of fucosterol (**2**) and 24-methylenecholesterol (**3**) (Table II). Thus both the activation energy for pathways A and C and the ionization potential of the side chain unsaturation is lowered by the C-28 methyl group of fucosterol (**2**) which leads to a greater selectivity for pathway A in **2** than **3**.

For the labeled 24-methylcholesta-5,24-dien-3β-ol (**46**) and the 27-norcholesta-5,24-dien-3β-ol (**57**) either pathway A or B appears equally feasible in terms of both radical-cation stability and the ring size for the hydrogen transfer. However, whereas **46** undergoes a partitioning between the two fragmentation pathways [*m/e* 314 (69%), *m/e* 271 (35%)], **57** gives almost exclusively *m/e* 271. This is probably partially the result of the higher ionization potential of the disubstituted unsaturation of **57** allowing the multistep higher activation energy process leading to the more stable mass 271 ion to compete more effectively with the McLafferty fragmentation and partially the result of the isomerization of the side chain double bond of **57** prior to fragmentation as discussed below.

On the basis of these arguments, 26,27-dinorcholesta-5,24-dien-3β-ol (**55**) might be expected to give some McLafferty fragmentation, but this is not the case. Here it is important to realize that the base peak in the spectrum is the molecular ion and that *m/e* 271 is of relatively low intensity (52%). Furthermore, high-resolution studies revealed that the *m/e* 271 fragment is a doublet (1:1) comprising a hydrocarbon fragment and an oxygen-containing fragment. The hydrocarbon fragment is probably derived by cleavage of ring A, a process observed in 3β-hydroxy-Δ^5-sterols such as cholesterol[18] and which becomes competitive here because of the higher ionization potential of the monosubstituted double bond. The oxygen-containing fragment is probably formed by an initial rearrangement of the terminal double bond to a more stable (substituted) position thus inhibiting a McLafferty fragmentation and causing the expected loss of side chain plus two hydrogens in the process characteristic of C-23 unsaturated sterols.[7] The presence of a relatively large *m/e* 55 fragment (90%) is consistent with such a rearrangement. There is ample precedent[19] for migrations of terminal olefins of this type prior to fragmentation, and indeed migrations of both cyclic[20] and acyclic nonterminal olefins[21] to a more highly substituted position have been observed in our laboratory. For example the C-25 unsaturated sterol stelliferasterol[21] (**62**) has fragments at *m/e* 328 and 314 which is most readily explained by a partial migration of the C-25(26) double bond prior to fragmentation as indicated in Scheme V. The mass 328 and 314 fragments are diagnostic of a C-25 unsaturated side chain sterol and have been useful in the structural elucidation of several novel marine sterols.[22] The initial partial migration of the double bond in other cases studied here cannot be excluded. Indeed such migrations may be responsible for the similarity in the spectra of the 24-methylenecholesterol (**34**) and the 24-methylcholesta-5,24-dien-3β-ol (**46**) and may also account for the relatively low intensity of *m/e* 314 in the case of the 24-norcholesta-5,25-dien-3β-ol (**49**) and the virtual absence of *m/e* 314 in **57**.

Scheme V



The dependence of the relative intensities of the mass 314 and 271 fragments upon the stereochemistry at C-20 (e.g., **34** vs. **35**; Table I) can be explained in terms of steric interactions in the transition state. The metastable defocusing data (Table II) for the two 24-methylenecholesterols **34** and **35** reveal that a larger proportion of the molecular ions in the first field free region fragment directly to *m/e* 271 in the (20*S*)-sterol **35** rather than the (20*R*)-sterol **34**. A consideration of molecular models in which we assume the ground state preferred conformation[23] for the side chain about the C-17(20) bond shows that the transition state for the McLafferty fragmentation of the 20*S* compound **35** has an unfavorable interaction between the C-18 methyl group and one of the C-24 methylene protons which is not present in the case of the 20*R* compound **34**. However, the transition state for the transfer of the proton from C-17 to C-28 via a seven-membered cyclic intermediate (pathway C), which leads to *m/e* 271, does not appear to be significantly affected by the C-20 stereochemistry. The McLafferty fragmentation is therefore more important in the 20*R* compound **34** than in the 20*S* compound **35**. Similar arguments apply to the other compounds in Table I. This dependence of the McLafferty fragmentation on the stereochemistry at C-20 is important in view of the recent isolation of several 20β-H sterols[11] and the anticipated emergence of numerous C-24 and C-25 unsaturated "extended" side chain sterols.[24]

(18) L. G. Partridge and C. Djerassi, *J. Org. Chem.*, **42**, 2799 (1977); S. G. Wyllie, B. Amos, and L. Tökés, *ibid.*, **42**, 725 (1977).

(19) F. Borchers, K. Levsen, H. Schwarz, C. Wesdemiotis, and H. U. Winkler, *J. Am. Chem. Soc.*, **99**, 6359 (1977), and references therein.

(20) L. G. Partridge, I. Midgley, and C. Djerassi, *J. Am. Chem. Soc.*, **99**, 7686 (1977).

(21) C. Djerassi, *Pure Appl. Chem.*, **50**, 171 (1978); N. Theobald, R. J. Wells, and C. Djerassi, *J. Am. Chem. Soc.*, in press.

(22) W. C. M. C. Kokke, W. H. Fenical, C. S. Pak, and C. Djerassi, *Tetrahedron Lett.*, in press.

(23) E. N. Trachtenberg, C. Byon, and M. Gut, *J. Am. Chem. Soc.*, **99**, 6145 (1977); W. Nes, T. E. Varkey, and K. Krevitz, *ibid.*, 260 (1977).

Experimental Section

General Notes. Low-resolution mass spectra and metastable defocusing data were obtained on an AEI MS-9 spectrometer operated in the direct inlet mode by Mr. R. G. Ross. High-resolution data were obtained on a Varian MAT 711 spectrometer equipped with a PDP-11/45 computer for data acquisition and processing and operated by Annemarie Wegmann.

The 60-MHz nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates T-60 NMR spectrometer, and the 100-MHz spectra were run on a Varian Associates XL-100 instrument by Dr. L. Durham. All spectra were recorded for $CDCl_3$ solutions with Me_4Si as internal standard. Infrared (IR) spectra were recorded on a Perkin-Elmer 700 infrared spectrophotometer, and ultraviolet (UV) spectra were determined on a Cary 14 recording spectrophotometer, using EtOH as solvent. Rotations were measured on a Perkin-Elmer 141 polarimeter, using chloroform solutions.

Melting points were determined on a Thomas-Hoover "Uni-Melt" capillary melting point apparatus and are uncorrected.

Column chromatography was performed on Merck TLC grade silica—PF-254.

Gas chromatography (GC) of all steroids was performed on U-shaped columns (1.8 m × 4-mm i.d.) packed with 1% OV-25 on 100–200 mesh Gas-Chrom Q. This column was mounted in a Hewlett-Packard 402 high-efficiency gas chromatograph equipped with a flame ionization detector. All injections were made at a column temperature of 255 °C, flash heater and detector temperatures of 270 °C, and a helium flow of 75 mL min^{-1}.

All solvents and reagents were purified as necessary before use according to standard procedures.

Microanalyses were performed in the microanalytical laboratory of the Department of Chemistry, Stanford University, by Mr. E. Meier and associates.

The phrase "work up by ether extraction" indicates that the reaction mixture was poured into water and extracted three times with ether. The combined extracts were washed sequentially with dilute hydrochloric acid and/or saturated sodium bicarbonate solution as necessary, water, and saturated sodium chloride solution and then were dried over anhydrous sodium sulfate and finally concentrated in vacuo to yield the product.

(20*R*)-20-Hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane-20-*d* (10) and (20*S*)-20-Hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane-20-*d* (11). The freshly prepared *i*-methyl ether aldehyde **7**[13] (1.6 g, 4.65 mmol) was added to a solution of sodium (0.8 g, 34.8 mg-atoms) in a mixture of MeOD (60 mL) and D_2O (10 mL). The homogeneous solution was stirred at 20 °C for 30 h under a nitrogen atmosphere. Addition of D_2O (30 mL) and removal of most of the methanol under reduced pressure gave a suspension which was extracted with ether. The combined extracts were washed with D_2O and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a colorless oil (1.6 g) which by TLC was mainly aldehyde. This crude product in anhydrous ether (25 mL) was added dropwise to an ice-cold solution of $LiAlH_4$ (0.2 g, 5.26 mmol) in anhydrous ether (25 mL). After 1 h, the excess of $LiAlH_4$ was destroyed by addition of a saturated aqueous sodium sulfate solution. The clear dry ethereal solution was filtered from the insoluble aluminum salts and concentrated under reduced pressure to give a colorless oil (1.5 g) which was chromatographed on silica gel (80 g), using hexane–ethyl acetate (9:1) as eluant.

Pure **10** (750 mg) was obtained as a glass: homogeneous by TLC and GC; $[\alpha]^{25}_D$ +42° (*c* 1.0); NMR (100 MHz) δ 3.77 and 3.43 (1 H each, d, *J* = 10 Hz, CH_2OH), 3.30 (3 H, s, OCH_3), 2.77 (1 H, br t, *J* = 3 Hz, C(**H**)OCH_3), 1.00 (3 H, s, C(19)-H_3), 0.93 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* (relative intensity) 347 (26), 332 (54), 315 (46), 292 (100); 96% isotopically pure d_1 [lit.[14] $[\alpha]^{25}_D$ + 41.8°].

Pure **11** (600 mg) was obtained as a viscous liquid which solidified on standing. A poorly crystalline sample was obtained from hexane: mp 85–86 °C; $[\alpha]^{25}_D$ +50° (*c* 1.0); NMR (100 MHz) δ 3.65 and 3.30 (1 H each, d, *J* = 10 Hz, CH_2OH), 3.30 (3 H, s, OCH_3), 2.77 (1 H, br t, *J* = 3 Hz, C(**H**)OCH_3), 1.03 (3 H, s, C(21)-H_3), 1.01 (3 H, s, C(19)-H_3), 0.73 (3 H, s, C(18)-H_3), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* 347 (25), 332 (50), 315 (43), 292 (100); 96% isotopically pure d_1 [lit.[14] mp 84.5–86 °C, $[\alpha]^{25}_D$ +51°].

Methyl (20*R*,22*E*)-6β-Methoxy-3α,5-cyclo-5α-chol-22-enate-20-*d* (12). Pyridine (0.56 mL, 7 mmol, freshly distilled from barium oxide) was added dropwise to a suspension of anhydrous chromium trioxide (0.35 g, 3.5 mmol) in anhydrous CH_2Cl_2 (30 mL). To the resultant burgundy solution, a solution of alcohol **11** (200 mg, 0.58 mmol) in CH_2Cl_2 (5 mL) was added in one portion. After 15 min the reaction mixture was diluted with ether and then filtered through Celite, and the black deposit was washed well with ether. The combined ethereal solutions were washed with dilute hydrochloric acid, water, and saturated sodium chloride solution and then dried over anhydrous sodium sulfate. Removal of the solvent gave a colorless oil which, according to TLC and GC, was mainly aldehyde **8**. The crude aldehyde **8** (190 mg, 0.55 mmol) in anhydrous dimethyl sulfoxide (Me_2SO) (15 mL) containing $(C_6H_5)_3P{=}CHCO_2CH_3$ (0.7 g, 2.1 mmol) was heated, under a nitrogen atmosphere, at 95 °C for 72 h. Work up by ether extraction and chromatography of the crude product on silica gel (40 g), using hexane–ether (9:1) as eluant, gave the α,β-unsaturated ester **12** (187 mg) as a colorless oil: homogeneous by TLC and GC; NMR (60 MHz) δ 6.78 (1 H, d, *J* = 16 Hz, C**H**=$CHCO_2CH_3$), 5.67 (1 H, d, *J* = 16 Hz, CH=C**H**CO_2CH_3), 3.67 (3 H, s, CO_2CH_3), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.07 (3 H, s, C(21)-H_3), 1.00 (3 H, s, C(19)-H_3), 0.73 (3 H, s, C(18)-H_3), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* 401 (34), 386 (51), 369 (55), 346 (100).

Methyl (20*S*,22*E*)-6β-Methoxy-3α,5-cyclo-5α-chol-22-enate-20-*d* (13). This compound was prepared from alcohol **10**, in the manner described above for **12**, in 67% overall yield. The ester **13** was obtained as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 6.89 (1 H, d, *J* = 16 Hz, C**H**=$CHCO_2CH_3$), 5.73 (1 H, d, *J* = 16 Hz, CH=C**H**CO_2CH_3), 3.72 (3 H, s, CO_2CH_3), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.00 (6 H, s, C(19)-H_3 and C(21)-H_3), 0.70 (3 H, s, C(18)-H_3), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* 401 (44), 386 (52), 369 (77), 346 (100).

Methyl (20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholanate-20-*d* (14). The unsaturated ester **12** (185 mg) in ethyl acetate (20 mL) was hydrogenated over platinum oxide (5 mg) at 18 °C under a slightly positive pressure of hydrogen for 9 h. Removal of the catalyst by filtration through Celite and concentration of the filtrate gave **14** (184 mg) as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 3.65 (3 H, s, CO_2CH_3), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C(**H**)OCH_3), 1.02 (3 H, s, C(19)-H_3), 0.92 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 403 (45), 388 (54), 371 (62), 348 (100).

Methyl (20*S*)-6β-methoxy-3α,5-cyclo-5α-cholanate-20-*d* (15) prepared by hydrogenation of the α,β-unsaturated ester **13** was obtained as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 3.67 (3 H, s, CO_2CH_3), 3.33 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C(**H**)OCH_3), 1.03 (3 H, s, C(19)-H_3), 0.83 (3 H, s, C(21)-H_3), 0.75 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 403 (50), 388 (56), 371 (74), 348 (100).

(20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholan-24-ol-20-*d* (16). The ester **14** (180 mg, 0.45 mmol) in anhydrous ether (15 mL) was treated, at 0 °C, with $LiAlH_4$ (30 mg, 0.8 mmol). After 15 min, destruction of the excess reducing agent and work up by ether extraction gave **16** as a colorless oil (160 mg) which crystallized slowly on standing: mp 95–97 °C; homogeneous by TLC and GC; NMR (60 MHz) δ 3.58 (2 H, br t, *J* = 6 Hz, C**H**$_2$OH), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.02 (3 H, s, C(19)-H_3), 0.92 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 375 (36), 360 (55), 343 (54), 320 (100).

(20*R*)-3β,24-Dihydroxychol-5-ene-20-*d* (18). The *i*-methyl ether **16** (5 mg) in 10% aqueous dioxane (2 mL) containing a catalytic amount of *p*-toluenesulfonic acid was heated under reflux for 1 h. The reaction mixture was diluted with water, and the crystalline product was removed by filtration. Recrystallization from aqueous methanol gave **18** as needles: mp 194–196 °C; NMR (100 MHz) δ 5.36 (1 H, br t, *J* = 5 Hz, C=C**H**CH$_2$), 3.60 (3 H, m, CH_2OH and CHOH), 1.01 (3 H, s, C(19)-H_3), 0.94 (3 H, s,

(24) T. H. Varkony, D. H. Smith, and C. Djerassi, *Tetrahedron*, 34, 841 (1978).

C(21)-H_3), 0.69 (3 H, s, C(18)-H_3) [lit.[25] mp 193–195 °C].

(20*S*)-6β-Methoxy-3α,5-cyclo-5α-cholan-24-ol-20-*d* (17). Reduction of the ester **15** (139 mg) with $LiAlH_4$ as described above gave **17** (120 mg) as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 3.63 (2 H, br t, J = 6 Hz, CH_2OH), 3.32 (3 H, s, OCH_3), 2.76 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.02 (3 H, s, C(19)-H_3), 0.83 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 375 (44), 360 (54), 343 (58), 320 (100).

(20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholest-24-ene-20-*d* (21). The alcohol **16** (160 mg, 0.43 mmol) in anhydrous dichloromethane (2 mL) was added in one portion to a solution of Collins' reagent[17] prepared from chromium trioxide (256 mg, 2.56 mmol) and pyridine (0.414 mL, 5.1 mmol) in anhydrous dichloromethane (10 mL). After 15 min work up as described above in the preparation of **12** gave the aldehyde **19** as a colorless oil (160 mg). Sodium hydride (103 mg of a 50% dispersion in oil, 2.2 mmol) was washed with hexane (two 5-mL portions) and then heated at 70 °C, under a nitrogen atmosphere with anhydrous Me_2SO (5 mL) for 0.5 h. The reaction mixture was cooled to 18 °C and a solution of isopropyltriphenylphosphonium iodide (926 mg, 0.21 mmol) in Me_2SO (5 mL) was added. The deep red solution was stirred for 15 min and then a solution of the aldehyde **19** in anhydrous tetrahydrofuran (THF) (5 mL) was added. The reaction mixture was stirred at 18 °C for 3 h. Work up by ether extraction and chromatography of the crude product on silica gel (15 g), using hexane–benzene (19:1) as eluant, gave the desired olefin **21** (128 mg) as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 5.00 (1 H, br t, J = 7 Hz, $CH{=}C(CH_3)_2$), 3.30 (3 H, s, OCH_3), 2.74 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.65 and 1.58 (3 H, br s, $CH{=}C(CH_3)_2$), 1.02 (3 H, s, C(19)-H_3), 0.92 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 399 (28), 384 (57), 367 (33), 352 (18), 344 (86), 253 (80), 69 (100).

(20*S*)-6β-Methoxy-3α,5-cyclo-5α-cholest-24-ene-20-*d* (22) was prepared in the manner described above from the alcohol **17** (126 mg) via the aldehyde **20** and was obtained as a colorless oil (104 mg): homogeneous by GC and TLC; NMR (60 MHz) δ 5.06 (1 H, br t, J = 7 Hz, $CH{=}C(CH_3)_2$), 3.30 (3 H, s, OCH_3), 2.74 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.65 and 1.58 (3 H, br s, $CH{=}C(CH_3)_2$), 1.02 (3 H, s, C(19)-H_3), 0.83 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 399 (18), 384 (42), 367 (15), 352 (14), 344 (59), 253 (100), 69 (76).

(20*R*)-Cholesta-5,24-dien-3β-ol-20-*d* (23). The *i*-methyl ether **21** (128 mg) in 10% aqueous dioxane (10 mL) containing *p*-toluenesulfonic acid (2 mg) was heated under reflux for 1 h. Addition of water gave **23** as a white solid. Recrystallization from aqueous methanol gave **23** as needles: mp 116–117 °C; $[\alpha]^{25}_D$ −40° (*c* 0.93); NMR (100 MHz) δ 5.36 (1 H, br d, J = 4.8 Hz, C(6)-H), 5.10 (1 H, br t, J = 7 Hz, $CH{=}C(CH_3)_2$), 3.51 (1 H, m, CHOH), 1.68 and 1.60 (3 H, br s, $HC{=}C(CH_3)_2$), 1.01 (3 H, s, C(19)-H_3), 0.93 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 385 (21), 370 (30), 352 (13), 301 (21), 299 (11), 271 (100), 253 (12), 69 (72); 95.4% isotopically pure d_1 compound [lit.[26] mp 117 °C, $[\alpha]^{25}_D$ −38.7°].

(20*S*)-Cholesta-5,24-dien-3β-ol-20-*d* (24). Hydrolysis of the *i*-methyl ether **22** as described above gave **24** as needles from aqueous methanol: mp 134–135 °C; $[\alpha]^{25}_D$ −31.9° (*c* 0.5); NMR (100 MHz) δ 5.36 (1 H, br d, J = 4.8 Hz, C(6)-H), 5.10 (1 H, br t, J = 7.0 Hz, $CH{=}C(CH_3)_2$), 3.50 (1 H, m, CHOH), 1.69 (3 H, d, J = 1 Hz) and 1.60 (3 H, br s) ($CH{=}C(CH_3)_2$), 1.01 (3 H, s, C(19)-H_3), 0.83 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 385 (47), 370 (23), 352 (7), 301 (16), 299 (5), 271 (100), 253 (11), 69 (53); 95.8% isotopically pure d_1 compound.

Anal. Calcd for $C_{27}H_{43}OD$: C, 84.17; H, 11.68. Found: C, 83.84; H, 11.48.

(20*R*,22*E*)-6β-Methoxy-3α,5-cyclo-5α-cholest-22-en-24-one-20-*d* (25). The aldehyde **8** (240 mg, 0.7 mmol) was treated with $(C_6H_5)_3P{=}CHCOCH(CH_3)_2$ (0.9 g, 2.6 mmol) in anhydrous Me_2SO (20 mL) under a nitrogen atmosphere at 95 °C for 48 h. Dilution with water (100 mL) and ether extraction gave an oil which was chromatographed on silica gel (20 g), using hexane–ether (9:1) as eluant, to afford the required enone **25** (140 mg) as an oil which solidified on standing. Recrystallization from aqueous methanol gave **25** as needles: mp 112–114 °C; UV λ_{max} 232 nm, ε 12 390; NMR (100 MHz) δ 6.73 (1 H, d, J = 16 Hz, $CH{=}CHCO$), 6.07 (1 H, d, J = 16 Hz, $CH{=}CHCO$), 3.32 (3 H, s, OCH_3), 2.77 (2 H, m, $CHOCH_3$ and $COCH(CH_3)_2$), 1.10 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.09 (3 H, s, C(21)-H_3), 1.03 (3 H, s, C(19)-H_3), 0.76 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 413 (30), 398 (52), 381 (42), 358 (100) [lit.[27] mp 115–116 °C; UV λ_{max} 228 nm, ε 15 000].

(20*S*,22*E*)-6β-Methoxy-3α,5-cyclo-5α-cholest-22-en-24-one-20-*d* (26). Prepared from the aldehyde **9** in the manner described above, **26** was obtained as a colorless oil: homogeneous by TLC and GC; NMR (60 MHz) δ 6.74 (1 H, d, J = 16 Hz, $CH{=}CHCO$), 6.03 (1 H, d, J = 16 Hz, $CH{=}CHCO$), 3.30 (3 H, s, OCH_3), 2.74 (2 H, m, $CHOCH_3$ and $COCH(CH_3)_2$), 1.08 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.00 (6 H, s, C(19)-H_3 and C(21)-H_3), 0.70 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 413 (25), 398 (45), 381 (37), 358 (100).

(20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholestan-24-one-20-*d* (27). The enone **25** (140 mg) in ethyl acetate (20 mL) was hydrogenated over platinum oxide (12 mg) under a slightly positive pressure of hydrogen at 18 °C for 14 h. Removal of the catalyst and solvent and chromatography of the crude product on silica gel (15 g), using hexane–ether (9:1) as eluant, gave the ketone **27** (112 mg) as plates from aqueous methanol: homogeneous by TLC but GC indicated an impurity (10%); mp 84–86 °C; NMR (100 MHz) δ 3.32 (3 H, s, OCH_3), 2.78 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.09 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.02 (3 H, s, C(19)-H_3), 0.90 (3 H, s, C(21)-H_3), 0.71 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 415 (47), 400 (53), 383 (50), 360 (100), 71 (79).

(20*S*)-6β-Methoxy-3α,5-cyclo-5α-cholestan-24-one-20-*d* (28) prepared by hydrogenation of **26** was obtained as an oil: homogeneous by TLC but only 90% pure by GC; NMR (60 MHz) δ 3.30 (3 H, s, OCH_3), 2.73 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.07 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.02 (3 H, s, C(19)-H_3), 0.80 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 415 (51), 400 (56), 383 (72), 360 (100), 71 (81). The compound was further characterized by acidic hydrolysis to afford **(20*S*)-3β-hydroxycholest-5-en-24-one-20-*d* (29)** as $CH(CH_3)_2$, from aqueous methanol: mp 139–140 °C; $[\alpha]^{25}_D$ − 51° (*c* 0.1); NMR (100 MHz) δ 5.35 (1 H, br d, J = 5 Hz, C(6)-H), 3.50 (1 H, br, CHOH), 1.09 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.00 (3 H, s, C(19)-H_3), 0.81 (3 H, s, C(21)-H_3), 0.70 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 401 (70), 386 (18), 383 (44), 368 (25), 314 (34), 271 (34), 43 (100).

Anal. Calcd for $C_{27}H_{43}O_2D$: C, 80.81; H, 11.21. Found: C, 81.04; H, 11.05.

(20*R*)-6β-Methoxy-24-methylene-3α,5-cyclo-5α-cholestane-20-*d* (30). Methyltriphenylphosphonium bromide (331 mg, 0.93 mmol) in dry benzene (8 mL) was treated with *n*-BuLi (0.93 mmol of a 1.2 N solution in hexane). The reaction mixture was heated under reflux for 1 h and then cooled to ambient temperature. A solution of the ketone **27** (95 mg, 0.22 mmol) in benzene (4 mL) was added and the reaction mixture heated under reflux for 2 h. Work up by ether extraction gave a yellow oil which was chromatographed on silica gel (15 g), using benzene–hexane (1:9) as eluant, to afford **30** as a colorless oil (84 mg): homogeneous by TLC and GC; NMR (100 MHz) δ 4.70 and 4.66 (1 H each, br, $C{=}CH_2$), 3.32 (3 H, s, OCH_3), 2.78 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.023 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.022 (3 H, s, C(19)-H_3), 0.935 (3 H, s, C(21)-H_3), 0.719 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 413 (42), 398 (36), 381 (34), 358 (100), 328 (30), 296 (19).

(20*S*)-6β-Methoxy-24-methylene-3α,5-cyclo-5α-cholestane-20-*d* (31) prepared from the ketone **28** (100 mg) in the manner described above was obtained as an oil (97 mg): homogeneous by GC and TLC; NMR (60 MHz) δ 4.70 and 4.65 (1 H each, br, $C{=}CH_2$), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, J = 3 Hz, $CHOCH_3$), 1.02 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 1.02 (3 H, s, C(19)-H_3), 0.85 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 413 (36), 398 (60), 381 (33), 366 (15), 358 (100), 328 (17), 296 (13).

(25) A. V. McIntosh, E. M. Meinzer, and R. H. Levin, *J. Am. Chem. Soc.*, **70**, 2955 (1948).

(26) U. H. M. Fagerlund and D. R. Idler, *J. Am. Chem. Soc.*, **79**, 6473 (1957).

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

(20*R*)-24-Methylenecholest-5-en-3β-ol-20-*d* (34). Acidic hydrolysis of the *i*-methyl ether **30** in the usual manner gave **34** as plates from aqueous methanol: mp 143–144 °C; $[\alpha]^{25}_D$ −36.4° (*c* 0.56); NMR (100 MHz) δ 5.35 (1 H, br d, *J* = 4 Hz, C(6)-H), 4.70 and 4.64 (1 H each, br, C=CH_2), 3.50 (1 H, m, CHOH), 1.023 (6 H, d, *J* = 7 Hz, $CH(CH_3)_2$), 1.010 (3 H, s, C(19)-H_3), 0.942 (3 H, s, C(21)-H_3), 0.684 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 399 (15), 384 (20), 381 (7), 366 (8), 314 (100), 299 (23), 296 (7), 271 (64); 92% isotopically pure d_1 compound [lit.[28] mp 142 °C $[\alpha]^{22}_D$ −35.0°].

(20*S*)-24-Methylenecholest-5-en-3β-ol-20-*d* (35) prepared by hydrolysis of the *i*-methyl ether **31** was obtained as needles from aqueous methanol: mp 133–135 °C; $[\alpha]^{25}_D$ −56.1° (*c* 0.53); NMR (100 MHz) δ 5.35 (1 H, br d, *J* = 4 Hz, C(6)-H), 4.72 and 4.67 (1 H each, br, C=CH_2), 3.50 (1 H, m, CHOH), 1.03 (6 H, d, *J* = 7 Hz, $CH(CH_3)_2$), 0.99 (3 H, s, C(19)-H_3), 0.85 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 399 (9), 384 (19), 381 (4), 366 (9), 314 (44), 299 (19), 296 (6), 271 (100); 90% isotopically pure d_1 compound.

Anal. Calcd for $C_{28}H_{45}OD$: 87.17; H, H, 11.77. Found: C, 84.17: H, 11.95.

(20*R*,24*Z*)-Stigmasta-5,24(28)-diene-3β-ol-20-*d* (36) was prepared from the ketone **27** by a Wittig reaction with ethyltriphenylphosphonium iodide in the manner described above to afford the *i*-methyl ether **32** which was hydrolyzed in the usual way to give **36** as needles from aqueous methanol: mp 134–135 °C; $[\alpha]^{25}_D$ −40° (*c* 0.5); NMR (100 MHz) δ 5.38 (1 H, br d, *J* = 5 Hz, C(6)-H), 5.13 (1 H, q, *J* = 7 Hz, C=C**H**CH_3), 3.50 (1 H, m, CHOH), 1.60 (3 H, d, *J* = 7 Hz, C=CHC**H**$_3$), 1.01 (3 H, s, C(19)-H_3), 0.98 (6 H, d, *J* = 7 Hz, $CH(CH_3)_2$), 0.94 (3 H, s, C-(21)-H_3), 0.69 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 413 (7), 398 (3), 395 (2), 314 (100), 299 (20), 281 (18), 271 (13); 81% isotopically pure d_1 compound [lit.[29] mp 135–136 °C, $[\alpha]_D$ −35.8°].

(20*S*,24*Z*)-Stigmasta-5,24(28)-dien-3β-ol-20-*d* (37) was obtained from the ketone **28** via the *i*-methyl ether **33** as described above as needles from aqueous methanol: mp 129–130 °C; $[\alpha]^{25}_D$ −62° (*c* 0.54); NMR (100 MHz) δ 5.35 (1 H, br d, *J* = 5 Hz, C(6)-H), 5.11 (1 H, q, *J* = 7 Hz, C=C**H**CH_3), 3.50 (1 H, m, CHOH), 1.59 (3 H, br d, *J* = 7 Hz, C=CHC**H**$_3$), 1.01 (3 H, s, C(19)-H_3), 0.98 (6 H, d, *J* = 6.2 Hz, $CH(CH_3)_2$), 0.84 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 413 (10), 398 (6), 314 (100), 299 (20), 296 (8), 281 (17), 271 (50); 94% isotopically pure d_1 compound.

Anal. Calcd for $C_{29}H_{47}OD$: C, 84.27; H, 11.83. Found: C, 84.28; H, 11.91.

(20*R*,22*E*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-22-en-24-one-20-*d* (38). The aldehyde **8** (190 mg, 0.55 mmol) in anhydrous Me_2SO (15 mL) was treated under a nitrogen atmosphere with Ph_3P=$CHCOCH_3$ (0.7 g, 2.2 mmol) at 95 °C for 72 h. Work up by ether extraction and purification by chromatography on silica gel (20 g), using hexane–benzene (9:1) as eluant, gave **38** (158 mg) as needles from aqueous methanol: mp 85–86 °C; UV λ_{max} 227 nm, ε 14 326; NMR (60 MHz) δ 6.62 (1 H, d, *J* = 16 Hz, C**H**=CHCO), 5.92 (1 H, d, *J* = 16 Hz, CH=C**H**CO), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 2.20 (3 H, s, $COCH_3$), 1.08 (3 H, s, C(21)-H_3), 1.02 (3 H, s, C(19)-H_3), 0.76 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 385 (26), 370 (53), 353 (59), 330 (100), 43 (96).

Anal. Calcd for $C_{26}H_{39}O_2D$: C, 81.20; H, 10.48. Found: C, 81.12; H, 10.48.

(20*S*,22*E*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-22-en-24-one-20-*d* (39) prepared from the aldehyde **9** in the manner described above was obtained as an oil: homogeneous by GC and TLC; NMR (60 MHz) δ 6.62 (1 H, d, *J* = 16 Hz, C**H**=CHCO), 5.91 (1 H, d, *J* = 16 Hz, CH=C**H**CO), 3.30 (3 H, s, OCH_3), 2.73 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 2.20 (3 H, s, $COCH_3$), 1.02 (6 H, s, C(21)-H_3 and C(19)-H_3), 0.70 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 385 (28), 370 (48), 353 (43), 330 (100), 43 (96).

(20*R*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholestan-24-one-20-*d* (40). The enone **38** (158 mg) in ethyl acetate (20 mL) was hydrogenated over platinum oxide (10 mg) at 18 °C for 9 h. Removal of the catalyst and solvent gave **40** (155 mg) as needles from aqueous methanol: mp 50–51 °C; NMR (60 MHz) δ 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 2.12 (3 H, s, $COCH_3$), 1.02 (3 H, s, C(19)-H_3), 0.90 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 387 (42), 372 (45), 355 (53), 332 (83), 43 (100).

Anal. Calcd for $C_{26}H_{41}O_2D$: C, 80.63; H, 11.10. Found: C, 80.85; H, 11.03.

Hydrolysis of **40** in the usual manner gave **(20*R*)-3β-hydroxy-26,27-dinorcholest-5-en-24-one-20-*d* (42)** as plates from aqueous methanol: mp 114–115 °C; NMR (100 MHz) δ 5.27 (1 H, br d, *J* = 4.8 Hz, C(6)-H), 3.42 (1 H, m, CHOH), 2.12 (3 H, s, $COCH_3$), 0.99 (3 H, s, C(19)-H_3), 0.91 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3) [lit.[30] mp 114–116 °C].

(20*S*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholestan-24-one-20-*d* (41). Hydrogenation of the enone **39** in the manner described above gave **41** as a colorless oil: homogeneous by TLC and GC; NMR (100 MHz) δ 3.32 (3 H, s, OCH_3), 2.77 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 2.13 (3 H, s, $COCH_3$), 1.02 (3 H, s, C-(19)-H_3), 0.81 (3 H, s, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 387 (44), 372 (41), 355 (61), 332 (74), 43 (100).

Hydrolysis of **41** in the usual manner gave **(20*S*)-3β-hydroxy-26,27-dinorcholest-5-en-24-one-20-*d* (43)** as plates from aqueous methanol: mp 172–173 °C; $[\alpha]^{25}_D$ −51° (*c* 0.1); NMR (100 MHz) δ 5.37 (1 H, br d, *J* = 6 Hz, C(6)-H), 3.50 (1 H, m, CHOH), 2.14 (3 H, s, $COCH_3$), 1.01 (3 H, s, C(19)-H_3), 0.81 (3 H, s, C-(21)-H_3), 0.69 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 373 (54), 358 (12), 355 (31), 340 (22), 314 (18), 213 (38), 43 (100).

Anal. Calcd for $C_{25}H_{39}O_2D$: C, 80.44; H, 10.98. Found: C, 80.61; H, 10.70.

(20*R*)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholest-24-ene-20-*d* (44). Sodium hydride (50 mg of a 50% dispersion in oil, 1.0 mmol) was washed with hexane (two 5-mL portions) and then heated under a nitrogen atmosphere at 70 °C with anhydrous Me_2SO (5 mL) for 0.5 h. The reaction mixture was cooled to 18 °C and a solution of isopropyltriphenylphosphonium iodide (419 mg, 1.0 mmol) in Me_2SO (5 mL) was added. The deep red solution was stirred for 15 min and then a solution of the ketone **40** (130 mg, 0.3 mmol) in anhydrous THF (5 mL) was added. The reaction mixture was stirred at 45 °C for 4 h. Work up by ether extraction and chromatography of the crude product on silica gel (15 g), using hexane–benzene (9:1) as eluant, gave **44** as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 3.30 (3 H, s, OCH_3), 2.72 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.60 (9 H, s, CH_3C=$C(CH_3)_2$), 1.01 (3 H, s, C(19)-H_3), 0.93 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 413 (22), 398 (34), 381 (24), 358 (63), 328 (26), 313 (13), 83 (100).

(20*S*)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholest-24-ene-20-*d* (45) was obtained from the ketone **41** in the manner described above as a colorless oil: homogeneous by TLC and GC; NMR (60 MHz) δ 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.60 (9 H, s, CH_3C=$C(CH_3)_2$), 1.03 (3 H, s, C(19)-H_3), 0.87 (3 H, s, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 413 (21), 398 (41), 381 (17), 366 (15), 358 (63), 328 (12), 83 (100).

(20*R*)-24-Methylcholest-24-en-3β-ol-20-*d* (46). Hydrolysis of **44** as described previously gave **46** as needles from aqueous methanol: mp 137–138 °C; $[\alpha]^{20}_D$ −44.9° (*c* 0.54); NMR (100 MHz) δ 5.35 (1 H, br d, *J* = 4.5 Hz, C(6)-H), 3.50 (1 H, br s, CHOH), 1.62 (9 H, s, CH_3C=$C(CH_3)_2$), 1.01 (3 H, s, C(19)-H_3), 0.96 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) *m/e* 399 (14), 384 (10), 381 (5), 314 (68), 299 (24), 271 (35), 83 (100); 90% isotopically pure d_1 compound.

Anal. Calcd for $C_{28}H_{45}OD$: C, 84.14; H, 11.85. Found: C, 83.70; H, 11.86.

(20*S*)-24-Methylcholest-24-en-3β-ol-20-*d* (47) was obtained by hydrolysis of **45**: mp 150–151 °C (aqueous methanol); $[\alpha]^{25}_D$ −61° (*c* 0.5); NMR (100 MHz) δ 5.35 (1 H, br d, *J* = 5 Hz, C(6)-H), 3.50 (1 H, br s, CHOH), 1.63 (9 H, s, CH_3C=$C(CH_3)_2$), 1.01 (3 H, s, C(19)-H_3), 0.86 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3);

(28) D. R. Idler and U. H. M. Fagerlund, *J. Am. Chem. Soc.*, **77**, 4142 (1955); **79**, 1988 (1957).

(29) K. Tsuda, K. Sakai, K. Tanabe, and Y. Kishida, *J. Am. Chem. Soc.*, **82**, 1442 (1960).

(30) E. Ayanoglu, C. Djerassi, T. R. Erdman, and P. J. Scheuer, *Steroids*, **31**, 815 (1978).

mass spectrum (70 eV) m/e 399 (15), 384 (18), 381 (2), 314 (29), 299 (11), 271 (100), 83 (71); 93% isotopically pure d_1 compound.

Anal. Calcd for $C_{28}H_{45}OD$: C, 84.14; H, 11.85. Found: C, 84.11; H, 11.70.

(20*S*)-24-Norcholesta-5,25-dien-24-ol-20-*d* (49). The ketone **41** (20 mg) was subjected to a Wittig reaction with methyltriphenylphosphonium bromide as described previously to afford (20*S*)-6β-methoxy-3α,5-cyclo-24-nor-5α-cholest-25-ene-20-*d* (**48**) as a colorless oil. Hydrolysis of **48** in the usual manner gave **49** (10 mg) as needles from aqueous methanol: mp 110–112 °C; $[\alpha]^{25}_D$ −36° (*c* 0.62); NMR (100 MHz) δ 5.34 (1 H, br d, *J* = 5 Hz, C(6)-H), 4.67 (2 H, br s, C=CH_2), 3.50 (1 H, br s, C**H**OH), 1.72 (3 H, br s, C=CCH_3), 1.00 (3 H, s, C(19)-H_3), 0.83 (3 H, s, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 371 (10), 356 (17), 353 (18), 314 (21), 271 (100).

Anal. Calcd for $C_{26}H_{41}OD$: C, 84.11; H, 11.58. Found: C, 84.00; H, 11.87.

(20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholan-24-ol (52). Methyl (20*R*)-3β-acetoxychol-5-enate (**50**) was treated with methanolic potassium hydroxide to afford methyl (20*R*)-3β-hydroxychol-5-enate as needles from aqueous methanol (mp 142–143 °C [lit.[31] mp 144 °C]) which was converted into methyl (20*R*)-6β-methoxy-3α,5-cyclo-5α-cholanate (**51**) by standard procedures. The *i*-methyl ether **51** was obtained as an oil: homogeneous by GC with R_f identical with that of **14**; NMR (60 MHz) δ 3.65 (3 H, s, CO_2CH_3), 3.30 (3 H, s, OCH_3), 2.75 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.02 (3 H, s, C(19)-H_3), 0.92 (3 H, d, *J* = 6 Hz, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3). Reduction of **51** with $LiAlH_4$ as described previously gave **52** as a colorless oil which crystallized slowly on standing: homogeneous by TLC and GC; other physical properties identical, except for the absence of deuterium, with those of **16**. The *i*-methyl ether **52** was further characterized by conversion into (20*R*)-3,24-dihydroxychol-5-ene; mp 194–196 °C (aqueous methanol); NMR (100 MHz) δ 5.36 (1 H, br d, *J* = 5 Hz, C(6)-H), 3.60 (3 H, m, C**H**OH and CH_2**O**H), 1.01 (3 H, s, C(19)-H_3), 0.94 (3 H, d, *J* = 6 Hz, C(21)-H_3), 0.69 (3 H, s, C(18)-H_3) [lit.[25] mp 193–195 °C].

(20*R*)-26,27-Dinorcholesta-5,24-dien-3β-ol (55). The *i*-methyl ether **52** (100 mg) was oxidized to the aldehyde **53** as described previously. The aldehyde **53** was subjected to a Wittig reaction with methyltriphenylphosphonium bromide to afford (20*R*)-6β-methoxy-3α,5-cyclo-25,26-dinor-5α-cholest-24-ene (**54**) as a colorless oil after chromatography on silica gel, using hexane–benzene (9:1) as eluant. Hydrolysis of **54** gave the dienol **55** as needles from aqueous methanol: mp 120–122 °C; $[\alpha]^{25}_D$ −37.4° (*c* 0.5); NMR (100 MHz) δ 5.76 (1 H, m, C**H**=CH_2), 5.52 (1 H, br d, *J* = 5 Hz, C(6)-H), 4.97 (1 H, br d, *J* = 17 Hz, CH=CH**H**), 4.92 (1 H, br d, *J* = 10 Hz, CH=CH**H**), 3.50 (1 H, br s, C**H**OH), 1.01 (3 H, s, C(19)-H_3), 0.94 (3 H, d, *J* = 6 Hz, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 356 (100), 341 (32), 338 (38), 323 (27), 314 (2), 271 (52), 245 (40), 213 (29), 55 (90).

Anal. Calcd for $C_{25}H_{40}O$: C, 84.28; H, 11.23. Found: C, 84.16; H, 11.23.

27-Norcholesta-5,24-dien-3β-ol (57). Ethyltriphenylphosphonium iodide (842 mg, 2 mmol) in anhydrous Me_2SO (5 mL) was added to a solution of dimsyl sodium prepared from sodium hydride (48 mg, 2 mmol) and anhydrous Me_2SO (10 mL). After 15 min a solution of the aldehyde **53** (200 mg, 0.5 mmol) in anhydrous THF (5 mL) was added and the reaction mixture stirred at 20 °C for 9 h. Work up by ether extraction and chromatography of the crude product on silica gel (12 g) with hexane–benzene (9:1) as eluant gave **(20*R*)-6β-methoxy-3α,5-cyclo-27-nor-5α-cholest-24-ene (56)** (150 mg) as a colorless oil: homogeneous by TLC and GC; NMR (60 MHz) δ 5.36 (2 H, m, C**H**=C**H**CH_3), 3.30 (3 H, s, OCH_3), 2.73 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.58 (3 H, d, *J* = 6 Hz, CH=CHC**H**$_3$), 1.03 (3 H, s, C(19)-H_3), 0.93 (3 H, d, *J* = 6 Hz, C(21)-H_3), 0.72 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 384 (40), 369 (60), 352 (45), 329 (100), 55 (88).

Hydrolysis of **56** in the usual manner gave **57** as needles from aqueous methanol: mp 122–123 °C; $[\alpha]^{25}_D$ −41.7° (*c* 0.85); NMR (100 MHz) δ 5.37 (3 H, m, C(6)-H and C**H**=C**H**CH_3), 3.50 (1 H, m, C**H**OH), 1.60 (3 H, d, *J* = 5.2 Hz, CH=CHC**H**$_3$), 1.01 (3 H, s, C(19)-H_3), 0.95 (3 H, d, *J* = 6.9 Hz, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 370 (20), 355 (34), 337 (17), 314 (2), 300 (19), 271 (100), 55 (94).

Anal. Calcd for $C_{26}H_{42}O$: C, 84.33; H, 11.34. Found: C, 84.54; H, 11.45.

24-Methyl-27-norcholesta-5,24-dien-3β-ol (61). The aldehyde **53** (100 mg) in anhydrous ether (20 mL) was treated at 0 °C with methyllithium (1 mL of a 1.5 N solution). After 1 h, destruction of the excess methyllithium by addition of water and concentration of the ethereal solution gave (20*R*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholestan-24-ol (**58**) (100 mg) as a colorless oil: homogeneous by GC and TLC; NMR (60 MHz) δ 3.76 (1 H, m, C**H**OH), 3.33 (3 H, s, OCH_3), 2.76 (1 H, br t, *J* = 3 Hz, C**H**OCH_3), 1.18 (3 H, d, *J* = 6 Hz, CH(OH)C**H**$_3$), 1.03 (3 H, s, C(19)-H_3), 0.91 (3 H, d, *J* = 6 Hz, C(21)-H_3), 0.73 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 388 (49), 373 (61), 356 (71), 333 (100).

Oxidation of **58** with Collins' reagent[17] gave (20*R*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholestan-24-one (**59**) whose physical properties were identical with those reported for **40** with the obvious exceptions due to the absence of deuterium. The ketone **59** (79 mg, 0.2 mmol) was subjected to a Wittig reaction with ethyltriphenylphosphonium iodide (351 mg, 0.8 mmol), using sodium hydride (20 mg, 0.8 mmol) and Me_2SO to generate the ylide. Work up by ether extraction and chromatography of the crude product on silica gel (10 g), using hexane–benzene (9:1) as eluant, gave 24-methyl-6β-methoxy-3α,5-cyclo-27-nor-5α-cholestane (**60**) (40 mg) as a colorless oil. Hydrolysis of **60** in the usual manner gave **61** as needles from aqueous methanol: mp 121-122 °C; $[\alpha]^{25}_D$ −33.7° (*c* 0.58); NMR (100 MHz) δ 5.34 (1 H, br d, *J* = 5 Hz, C(6)-H), 5.16 (1 H, q, *J* = 5.2 Hz, C=C**H**CH_3), 3.50 (1 H, m, C**H**OH), 1.58 (3 H, s, C**H**$_3$C=CHCH_3), 1.55 (3 H, d, *J* = 5.2 Hz, CH_3C=CHC**H**$_3$), 1.01 (3 H, s, C(19)-H_3), 0.94 (3 H, d, *J* = 5.2 Hz, C(21)-H_3), 0.68 (3 H, s, C(18)-H_3); mass spectrum (70 eV) m/e 384 (15), 369 (8), 366 (3), 314 (100), 299 (33), 281 (20), 271 (23), 69 (56).

Anal. Calcd for $C_{27}H_{44}O$: C, 84.38; H, 11.45. Found: C, 84.28; H, 11.81.

Acknowledgments. We thank the Upjohn Corp. and Ciba-Geigy for donations of stigmasterol and of methyl 3β-acetoxycholenate, respectively. Financial support by the National Institutes of Health[2] is gratefully acknowledged.

Registry No. **1**, 313-04-2; **2**, 17605-67-3; **3**, 474-63-5; **7**, 25819-77-6; **8**, 70209-13-1; **9**, 70209-14-2; **10**, 70209-15-3; **11**, 70209-16-4; **12**, 70209-17-5; **13**, 70266-88-5; **14**, 70209-18-6; **15**, 70266-89-6; **16**, 70209-19-7; **17**, 70266-90-9; **18**, 70209-20-0; **19**, 70209-21-1; **20**, 70266-91-0; **21**, 70209-22-2; **22**, 70266-92-1; **23**, 70209-23-3; **24**, 70266-93-2; **25**, 70209-24-4; **26**, 70266-94-3; **27**, 70209-25-5; **28**, 70266-95-4; **29**, 70209-26-6; **30**, 70209-27-7; **31**, 70266-96-5; **32**, 70267-02-6; **33**, 70209-28-8; **34**, 70209-29-9; **35**, 70266-97-6; **36**, 70209-30-2; **37**, 70266-98-7; **38**, 70209-31-3; **39**, 70266-99-8; **40**, 70209-32-4; **41**, 70267-00-4; **42**, 70209-33-5; **43**, 70267-70-8; **44**, 70286-00-9; **45**, 70209-34-6; **46**, 70209-35-7; **47**, 70267-01-5; **48**, 70224-68-9; **49**, 70209-36-8; **50**, 31823-53-7; **51**, 13223-95-5; **52**, 13223-96-6; **53**, 70209-37-9; **54**, 70209-38-0; **55**, 70209-39-1; **56**, 70209-40-4; **57**, 70209-41-5; **58**, 70209-42-6; **59**, 68150-96-9; (*E*)-**60**, 70209-43-7; (*Z*)-**60**, 70209-45-9; (*E*)-**61**, 70209-44-8; (*Z*)-**61**, 70209-46-0; 3-methyl-1-(triphenylphosphoranylidene)-2-butanone, 19753-67-4; ethyltriphenylphosphonium iodide, 4736-60-1; 1-(triphenylphosphoranylidene)-2-propanone, 1439-36-7; isopropyltriphenylphosphonium iodide, 1530-33-2; methyltriphenylphosphonium bromide, 1779-49-3; 3β,24-dihydroxychol-5-ene, 54668-67-6; ethylidenetriphenylphosphorane, 1754-88-7.

(31) R. Schoenheimer and F. Berliner, *J. Biol. Chem.*, **115**, 19 (1936).