Mass Spectrometry in Structural and Stereochemical Problems. CCXXX.[1] Preparation of 5α,20α- and 5α,17α,20α-Cholestane-3β,6α-diol. Electron Impact Induced Fragmentation of Steroidal $\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$ Olefins

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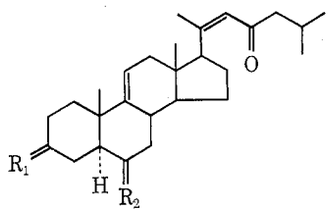
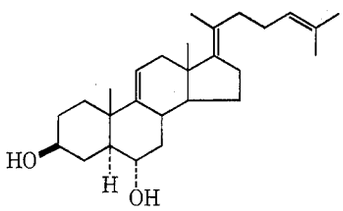
To find convenient ways to identify $\Delta^{17(20)}$ and $\Delta^{20(22)}$ steroidal olefins related to marine natural products **1**, **2**, and **3**, our paper describes relevant synthesis of parent and deuterated compounds as well as nmr and mass spectral criteria for their differentiation. Also described is the preparation of 5α,20α-cholestane-3β,6α-diol (**7**) and 5α,17α,20α-cholestane-3β,6α-diol (**10**).

In recent reports[2] on the isolation and structure determination of the star fish genins 5α-cholesta-9(11),20(22)-diene-3β,6α-diol-23-one (**1a**), 5α-cholesta-9(11),17(20),24-triene-3β,6α-diol (**2**), and 24ξ-methyl-5α-cholesta-9(11),20(22),diene-3β,6α-diol (**3**) we indicated that hydrogenation (10% palladium on charcoal) of **1** furnished 5α-cholestane-3β,6α-diol-23-one (**4**, 20%) and an unknown isomeric keto diol **5** (80%). The keto diol **5** was assigned the epimeric configuration at C-20 because its Cotton effect was opposite to that of compound **4**. Wolff–Kishner reduction of **4** and **5** furnished 5α-cholestane-3β,6α-diol (**6**) and the unknown isomeric diol **7**, respectively. Oxidation of **6** and **7** provided two isomeric diones (**8** and **9**) which depicted similar spectral characteristics (ir, nmr, mass spectra, CD, and ORD) but differed substantially in their melting points and gas chromatographic mobilities.



1a, R_1 = 3β-OH; R_2 = 6α-OH

1b, R_1 = 3β-OAc; R_2 = 6α-OAc



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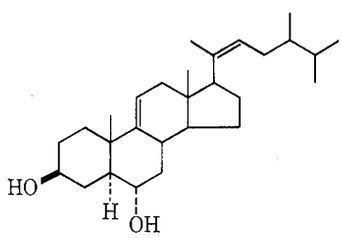

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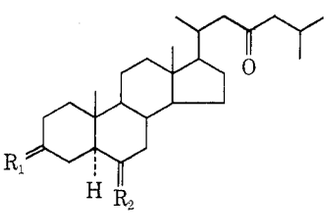
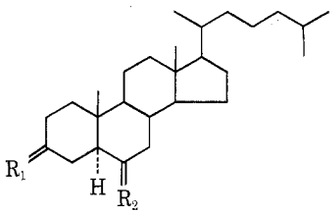


4, R_1 = 3β-OH; R_2 = 6α-OH

4a, R_1 = 3β-OAc; R_2 = 6α-OAc

5, R_1 = 3β-OH; R_2 = 6α-OH (20α)

5a, R_1 = 3β-OAc; R_2 = 6α-OAc (20α)



6, R_1 = 3β-OH; R_2 = 6α-OH (20β)

6a, R_1 = 3β-OAc; R_2 = 6α-OAc (20β)

7, R_1 = 3β-OH; R_2 = 6α-OH (20α)

7a, R_1 = 3β-OAc; R_2 = 6α-OAc (20α)

7b, $R_1 = R_2 = H_2$ (20α)

8, $R_1 = R_2 = O$ (20β)

9, $R_1 = R_2 = O$ (20α)

10, R_1 = 3β-OH; R_2 = 6α-OH (17,20α)

10a, R_1 = 3β-OAc; R_2 = 6α-OAc (17,20α)

11, $R_1 = R_2 = O$ (17,20α)

Examination of the literature indicated the existence of a considerable void concerning the spectral behavior[3]

(1) For preceding article, see S. Hammerum and C. Djerassi, *Org. Mass Spectrom.*, in press.

(2) Y. M. Sheikh, B. M. Tursch, and C. Djerassi, *J. Amer. Chem. Soc.*, **94**, 3278 (1972); Y. M. Sheikh, B. M. Tursch, and C. Djerassi, *Tetrahedron Lett.*, 3721 (1972).

(3) N. K. Chaudhuri, R. Nicholson, J. G. Williams, and M. Gut, *J. Org. Chem.*, **34**, 3767 (1969), have recently described a new synthesis of cholesterol and related C_{27} steroids which includes the preparation and properties of *cis*-$\Delta^{17(20)}$- and -$\Delta^{20(22)}$-cholesterol.

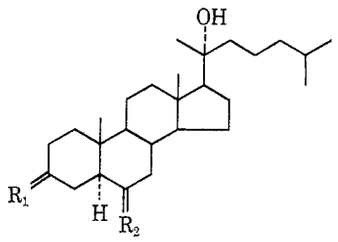
associated with the unusual $\Delta^{17(20)}$ and $\Delta^{20(22)}$ olefinic linkages in genins **1**, **2**, and **3**. The present work therefore was aimed toward (a) preparation of $\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$ steroidal olefins; (b) accumulation of nmr and gas chromatographic data thereby arriving at some parameters which could perhaps be used for structure elucidation of similar natural products, notably from marine sources; and (c) a detailed study of the mass spectrometric fragmentation of these olefins, thus supplementing the already available data on sterols with unsaturated side chains; and (d) finally to prepare 5α,20α-cholestane-3β,6α-diol (**7**) and 5α,17α,20α-cholestane-3β,6α-diol (**10**) so as to confirm the sterochemical assignment of **5**.

The $\Delta^{17(20)}$ olefinic linkage is a necessary feature[4] for bioactivity of the fungal metabolites of the fusidic acid and cephalosporin series. Presently no convenient way is available to introduce this unusual functionality. $\Delta^{20(22)}$-Unsaturated steroids can be obtained by dehydration of 22-hydroxy steroids[5] which can be prepared conveniently by a method developed by Cole and Julian[6] involving a Grignard reaction with bisnorcholenic acid derivatives. This method has been extensively used for the synthesis of naturally occurring steroids including cholesterol,[7] dihydrobrassicasterol,[8] campesterol,[9] ecdysone,[10] and 20-hydroxyecdysones.[11] More recently $\Delta^{20(22)}$ steroids, obtained by dehydration of 20-hydroxy precursors, have served as intermediates in synthesis of β-sitosterol[12] and bufadienolides.[13] Whereas the preparation of $\Delta^{20(22)}$ olefins by Wittig reaction generally results in poor yield,[14] the $\Delta^{20(21)}$ olefinic linkage has been introduced[15] by Wittig reaction of 21-nor-20-keto steroids (easily accessible from the corresponding etioallocholanic acid derivatives and organocadmium derivatives) and it, in turn, provides a facile entry[16] into 21-functionalized steroids of the 20*S* (α) configuration.

For our purpose we employed the method of Woodward, *et al.*,[17] which involves the reaction of appropriate 20-keto steroids with alkylmagnesium halide to furnish 20α-hydroxy steroids[17b] which after appropriate protection of nuclear functionalities can be dehydrated under a variety of conditions. The resulting olefins can then be separated by chromatography over silver nitrate impregnated silica gel.

The required starting ketone 5α-pregnane-3β,6α-diol-20-one diacetate[18] was prepared by hydroboration of Δ^5-pregnenolone acetate 20-ethylene ketal,[19] followed by reacetylation of the hydroxyl functions and acid-catalyzed cleavage of the ketal. Treatment of this ketone or of Δ^5-pregnenolone acetate with the Grignard complex of 1-bromo-4-methylpentane or 1-bromo-4-methyl-3-pentene (prepared by reaction of hydrobromic acid with cyclopropyldimethylcarbinol[20]) according to the general procedure of Petrow and Stuart-Webb[21] furnished the desired 20-hydroxy steroids (**12**–**15**). The expected 20α stereochemistry of the resulting carbinols was confirmed by nmr spectroscopy.[22]

Dehydration of the 20-hydroxylated steroids (**12**–**15**) gave varying proportions of the isomeric olefins



12, R_1 = 20α-OH, H; R_2 = H (Δ^5)

12a, R_1 = 20α-OAc, H; R_2 = H (Δ^5)

13, R_1 = 20α-OH, H; R_2 = H (Δ^5,Δ^{24})

13a, R_1 = 20α-OAc, H; R_2 = H (Δ^5,Δ^{24})

14, R_1 = 20α-OH, H; R_2 = 22-OH, H

14a, R_1 = 20α-OAc, H; R_2 = 22-OAc, H

14b, R_1 = R_2 = O

15, R_1 = 20α-OH, H; R_2 = 22-OH, H (Δ^{24})

15a, R_1 = 20α-OAc, H; R_2 = 22-OAc, H (Δ^{24})

($\Delta^{17(20)}$, $\Delta^{20(21)}$, $\Delta^{20(22)}$) depending on the reaction conditions (see Table I). It is important to note that our results differ from those reported for 20-hydroxycholanic acid derivatives[13] (**16**) [$SOCl_2$/Py → 80%

(4) For structure activity study, see W. O. Gotfredsen, W. von Daehne, L. Tybring, and S. Vangedol, *J. Med. Chem.*, **9**, 15 (1966). Recently the $\Delta^{17(20)}$ double bond has been introduced into tumulosic acid: I. L. Batey, J. T. Pinhey, B. J. Ralph, J. J. H. Simes, and M. Wootton, *J. Chem. Soc., Perkin Trans.* **1**, 2260 (1972).

(5) K. Tsuda and R. Hyatsu, *J. Amer. Chem. Soc.*, **81**, 5987 (1959); L. F. Fieser and W. Y. Huang, *ibid.*, **75**, 5356 (1953).

(6) W. Cole and P. L. Julian, *J. Amer. Chem. Soc.*, **67**, 1369 (1945).

(7) A. Romeo and R. Villotti, *Ann. Chim. (Rome)*, **47**, 618 (1957).

(8) A. Martinez, A. Romeo, and V. Tortorella, *Gazz. Chim. Ital.*, **97**, 96 (1967).

(9) G. Tarzia, V. Tortorella, and A. Romeo, *Gazz. Chim. Ital.*, **97**, 102 (1967).

(10) J. B. Siddall, J. P. Marshal, A. Bowers, A. D. Cross, J. A. Edwards, and J. H. Fried, *J. Amer. Chem. Soc.*, **88**, 379 (1966); J. B. Siddall, A. D. Cross, and J. H. Freid, *ibid.*, **88**, 862 (1966); R. Wiechert, A. Furlenmeier, A. Furst, A. Langemann, and G. Waldvogel, *Helv. Chim. Acta*, **49**, 1601 (1966).

(11) G. Huppi and J. B. Sidall, *J. Amer. Chem. Soc.*, **89**, 6790 (1967).

(12) R. Ikan, A. Markus, and E. D. Bergman, *J. Org. Chem.*, **36**, 3944 (1971).

(13) S. Sarel, Y. Shalon, and Y. Yanuka, *Chem. Commun.*, 80 (1970).

(14) Unpublished results from this laboratory. Recently ylides prepared from methoxymethylphosphorane have been treated with 20-oxopregnanes [see G. R. Pettit, B. Green, G. L. Dunn, and P. Sander-Plassman, *J. Org. Chem.*, **35**, 1385 (1970)].

(15) F. Sondheimer and R. Mechoulam, *J. Amer. Chem. Soc.*, **80**, 3087 (1959).

(16) J. Bottin and M. Fetizon, *Chem. Commun.*, 1087 (1971).

(17) (a) R. B. Woodward, F. Sondheimer, D. Taube, K. Heusler, and W. M. McLamore, *J. Amer. Chem. Soc.*, **74**, 4223 (1952). (b) N. K. Chaudhri, J. Williams, R. Nickolson, and M. Gut, *J. Org. Chem.*, **34**, 3759 (1969), have recently established the stereochemistry of the intermediates 20-carbinol.

(18) J. Gurst, Y. M. Sheikh and C. Djerassi, *J. Amer. Chem. Soc.*, **95**, 638 (1973).

(19) W. J. Adams, D. K. Patel, V. Petrow, I. A. Stuart-Webb, and B. Sturgeon, *J. Chem. Soc.*, 4490 (1956).

(20) M. Julia, S. Julia, and R. Guegan, *Bull. Soc. Chim. Fr.*, 1072 (1960).

(21) V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 4675 (1956).

(22) E. J. Middleton, D. H. S. Horn, and M. N. Galbraith, *Austral. J. Chem.*, **25**, 1245 (1972).

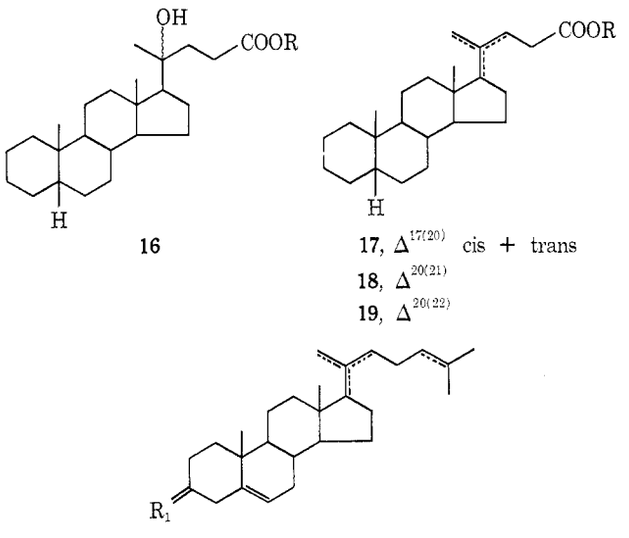
TABLE I

PRODUCT YIELDS[a] OF VARIOUS OLEFINS ON DEHYDRATION OF 20-HYDROXY STEROIDS (12a, 13a, 14a, AND 15a)

Compd	% dehydration	Method	Products (%) $\Delta^{17(20)}$	$\Delta^{20(22)}$	$\Delta^{20(21)}$
12a	100	$SOCl_2$/Py	20a (11)	22a (44)	21a (44)
	70	$POCl_3$/Py	20a (29)	22a (48)	21a (23)
13a	100	$SOCl_2$/Py	23a (16)	25a (28)	24a (52)
	80	$POCl_3$/Py	23a (31)	25a (31)	24a (31)
14a	100	$SOCl_2$/Py	26a (14)	28a (40)	27a (45)
	85	$POCl_3$/Py	26a (28)	28a (44)	27a (28)
		$CH_3CH_2CO_2H$, 12 hr	26a (10–15)	28a (80)	27a (<5)
15a	100	$SOCl_2$/Py	29a (15)	31a (23)	30a (54)
	85	$POCl_3$/Py	29a (30)	31a (34)	30a (36)

[a] Isolated yields.

$\Delta^{17(20)}$-17 (cis + trans); $POCl_3$/Py → principally $\Delta^{20(22)}$-19]. These results have recently been explained[23] by invoking thermodynamic control in the former ($SOCl_2$) and kinetic control in the latter ($POCl_3$) reaction.



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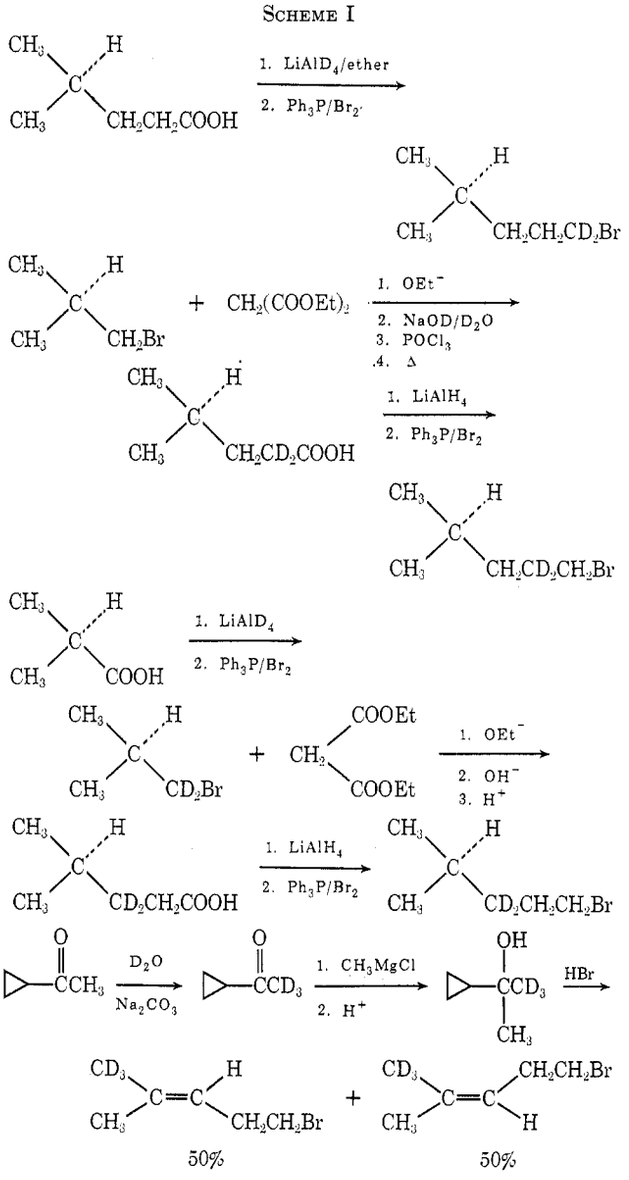
17, $\Delta^{17(20)}$ cis + trans
18, $\Delta^{20(21)}$
19, $\Delta^{20(22)}$

20, R_1 = 3α-OH ($\Delta^{17(20)}$)
20a, R_1 = 3α-OAc ($\Delta^{17(20)}$)
21, R_1 = 3α-OH ($\Delta^{20(21)}$)
21a, R_1 = 3α-OAc ($\Delta^{20(21)}$)
22, R_1 = 3α-OH ($\Delta^{20(22)}$)
22a, R_1 = 3α-OAc ($\Delta^{20(22)}$)
23, R_1 = 3α-OH ($\Delta^{17(20)}$,Δ^{24})
23a, R_1 = 3α-OAc ($\Delta^{17(20)}$,Δ^{24})
24, R_1 = 3α-OH ($\Delta^{20(21)}$,Δ^{24})
24a, R_1 = 3α-OAc ($\Delta^{20(21)}$,Δ^{24})
25, R_1 = 3α-OH ($\Delta^{20(22)}$,Δ^{24})
25a, R_1 = 3α-OAc ($\Delta^{20(22)}$,Δ^{24})

The structures of the resulting olefins were established by a combination of ir, nmr, and high resolution mass spectral measurements. The $\Delta^{20(21)}$ olefins depict a characteristic doublet around δ 4.70–4.90 due to the vinylic methylene protons, whereas the 22 vinylic proton in $\Delta^{20(22)}$ olefins appears as a triplet at 5.10 (J = 6 Hz). From the chemical shifts of the various $\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$ model olefins (see Table II) it was possible to deduce shift parameters for the C-18 and C-19 methyls. Introduction of a $\Delta^{17(20)}$ linkage results in a downfield shift of the C-18 methyl signal by δ 0.17 ± 0.01, whereas both $\Delta^{20(21)}$ and $\Delta^{21(22)}$ double bonds shift the C-18 protons upfield by a value of 0.11 and 0.13, respectively. The C-19 methyl signal remains essentially unaffected in either case. The validity of these parameters, which

SCHEME I



(23) D. N. Kirk in Specialists Periodical Report, "Terpenoids and Steroids," Vol. 1, The Chemical Society, London, 1970, pp 289, 290.

TABLE II

NUCLEAR MAGNETIC RESONANCE DATA

Compd		C-18		C-19		C-21		C-26,27		Olefinic protons	
Parent	Acetate	Parent	Acetate	Parent	Acetate	Parent	Acetate	Parent	Acetate	Parent	Acetate
6 (20β)		0.65		0.81		0.90		0.86			
7 (20α)		0.65		0.81		0.90		0.86			
10 (17α,20α)		0.65		0.81		0.90		0.86			
12	**12a**		0.86		1.03		1.26		0.86		5.36
13	**13a**	0.86	0.85	1.00	1.00	1.28	1.26	1.60	1.63	5.10	5.10
								1.66	1.66	5.33	5.35
14	**14a**	0.81	0.82	0.83	0.86	1.24	1.23	0.86	0.85		
15	**15a**	0.81	0.83	0.83	0.88	1.25	1.25	1.62	1.60	5.13	5.08
									1.65		
20[a]	**20a**	0.86		1.01		1.68		0.88		5.36	
21	**21a**	0.58	0.58	1.00	1.01			0.83	0.89	4.76, 4.86	4.76, 4.86
										5.33	5.33
22	**22a**	0.55		1.01		1.63		0.88		5.33–5.41	
23	**23a**	0.85	0.86	1.01	1.00	1.60 or 1.68	1.60 or 1.68	1.60 or 1.68	1.60 or 1.68	5.15	5.13
										5.38	5.36
24	**24a**	0.56	0.58	1.00	1.00			1.60	1.60	4.78, 4.86	4.76, 4.83
								1.66	1.66	5.10, 5.33	5.10, 5.36
25	**25a**		0.55		1.03		1.63 or 1.68		1.63		5.15
									1.68		5.35
26	**26a**	0.84	0.83	0.84	0.90	1.68	1.66	0.87	0.87		
27	**27a**		0.55		0.87				0.86		4.76
											4.85
28	**28a**	0.51	0.50	0.83	0.87	1.60	1.62	0.86	0.88	5.13	5.13
29	**29a**	0.81	0.82	0.81	0.87	1.60 or 1.66	1.60 or 1.66	1.60 or 1.66	1.60 or 1.66	5.13	5.13
30	**30a**	0.55	0.56	0.80	0.89			1.60 or 1.66	1.62 or 1.70	4.78, 4.85	4.78, 4.88
										5.10	5.10
31	**31a**		0.53		0.90		1.63		1.63 or 1.66		5.10

[a] See ref 3.

should be useful in the identification of marine sterols, is supported by the good agreement of calculated and observed chemical shifts for the angular methyl groups in genin **2** (calculated for C-18 0.75 and C-19 0.94, observed 0.72 and 0.94) and genin **3** (calculated for C-18 0.48 and C-19 1.01, observed 0.49 and 1.01).

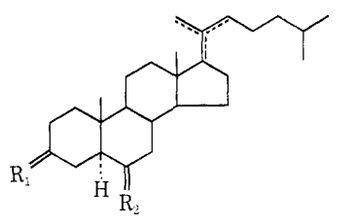
Deuterated analogs of the $\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$ olefins required for the mass spectrometric analysis were obtained by dehydration of the labeled 20-hydroxy steroids which in turn were prepared as described above but substituting the appropriately deuterated Grignard reagents. The labeled reagents were prepared by the methods outlined in Scheme I.

Hydrogenation (10% Pd/C, EtOAc) of $\Delta^{20(21)}$-cholestene-3β,6α-diol (**27a**) and $\Delta^{20(21),24}$-cholestadiene-3β,6α-diol diacetates (**30a**) furnished the 20β- and 5α,20α-cholestane-3β,6α-diol diacetates (**6a** and **7a**) which on subsequent saponification provided the parent diols (**6** and **7**). Similar hydrogenation of **26a** and **29a** followed by saponification furnished the normal and 17α,20α-diols (**6** and **10**). Jones oxidation of **7** and **10** gave the corresponding diketones (**9** and **11**). Identity of the 20α-diol **7** and the diketone **9** with those derived from the natural starfish genin **1** could be established by spectral comparison (ir, nmr, mass spectra, and gc) and mixture melting point determination.

The C-20 isomeric nature of **6** could be established independently by comparison (gc, mass spectra) of hydrocarbons derived from the diketone **9** and 20α-cholestanol.[24]

Discussion of Mass Spectra

The mass spectra of steroidal $\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$ olefins are shown in Figures 1–5. Although they bear a superficial resemblance to the analogous



26, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{17(20)}$)

26a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{17(20)}$)

27, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{20(21)}$)

27a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{20(21)}$)

28, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{20(22)}$)

28a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{20(22)}$)

28b, $R_1 = R_2 = O$ ($\Delta^{20(22)}$)

29, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{17(20)}$,Δ^{24})

29a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{17(20)}$,Δ^{24})

30, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{20(21)}$,Δ^{24})

30a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{20(21)}$,Δ^{24})

31, R_1 = 20β-OH; R_2 = 17α-H ($\Delta^{20(22)}$,Δ^{24})

31a, R_1 = 20β-OAc; R_2 = 17α-H ($\Delta^{20(22)}$,Δ^{24})

(24) G. V. Nair and E. Mosettig, *J. Org. Chem.*, **27**, 4659 (1962).

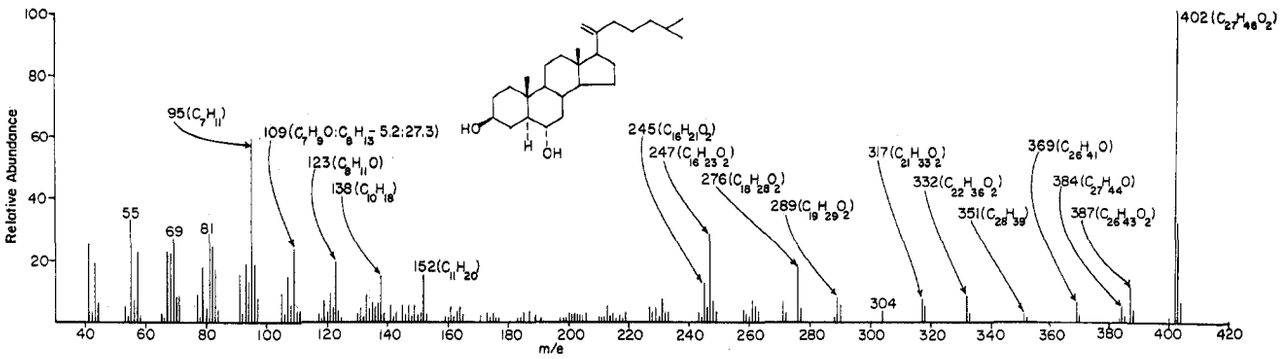


Figure 1.—Mass spectrum (70 eV) of 5α-cholest-20(21)-ene-3β,6α-diol (**27**).

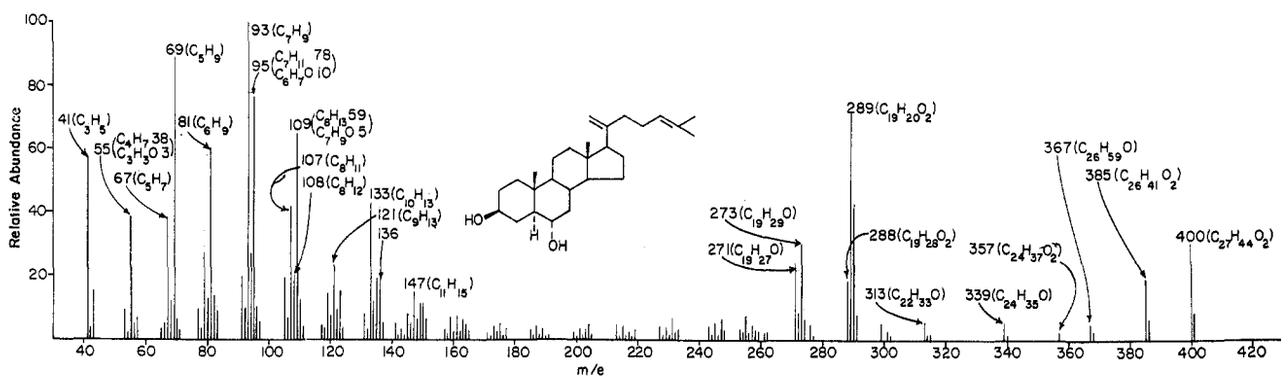


Figure 2.—Mass spectrum (70 eV) of 5α-cholesta-20(21),24-diene-3β,6α-diol (**30**).

TABLE III

SHIFTS OF THE PRINCIPAL PEAKS, *m/e* (RELATIVE INTENSITIES), IN THE MASS SPECTRA (70 eV) OF DEUTERATED 5α-CHOLEST-20(21)-ENE-3β,6α-DIOL (**27**)

Compd	Isotopic purity	M⁺ (%)	*m/e* (%)					
27 (d_0)		402 (100)	332 (9)	276 (17)	247 (30)	152 (15)	138 (15)	95 (57)
								96 (17)
								97 (5)
27b (17,21,21-d_3)	94% d_3	405 (100)	335 (13)	276 (17)	247 (46)	155 (20)	141 (9)	95 (45)
	4% d_2							96 (19)
	2% d_1							97 (25)
								98 (23)
27c (22-d_2)	93% d_2	404 (100)	334 (12)	276 (15)	247 (40)	154 (22)	140 (5)	95 (67)
	6% d_1							96 (21)
	1% d_0							97 (27)
27d (23-d_2)	91% d_2	404 (100)	332 (10)	276 (15)	247 (31)	154 (15)	140 (6)	95 (45)
	7% d_1							96 (15)
	2% d_0							97 (31)
27e (24-d_2)	85% d_2	404 (100)	333 (6.0)	276 (17)	247 (41)	154 (21)	140 (7)	95 (73)
	8% d_1		332 (6.0)					96 (20)
	7% d_0							97 (7)
27f (26-d_3)	50% d_3		332 (9.0)	276 (—)	247 (31)			
	20% d_2							
	15% d_1							

Δ^{22}, Δ^{23}, and Δ^{24} sterols,[25] they display marked differences from those observed for sterols possessing a saturated side chain.[26]

$\Delta^{20(21)}$ Olefins (27) (Figure 1).—The peak shifts found in the various labeled $\Delta^{20(21)}$ steroids are summarized in Table III.

The spectrum (Figure 1) of the $\Delta^{20(21)}$ olefin **27** is characterized by an intense molecular ion (base peak), a complete absence of allylic fission, and a weak "McLafferty ion" of mass 332 arising (Scheme II) both *via* six- and seven-membered transition states.

Minor peaks at *m/e* 290 and 289 are generated by loss of the side chain with transfer of one and two hydrogens. Since deuterium labeling indicated the complete loss of the C_{17} H, it is likely that this fragmentation corresponds to that elucidated[25] earlier in Δ^{23} and Δ^{24} steroids. However the peak at *m/e* 276 (Figure 1), involving loss of the side chain in addition to C_{17}, has no precedent among Δ^{23} and Δ^{24} steroids.[25]

(25) S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
(26) J. Karliner, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **31**, 710 (1966).

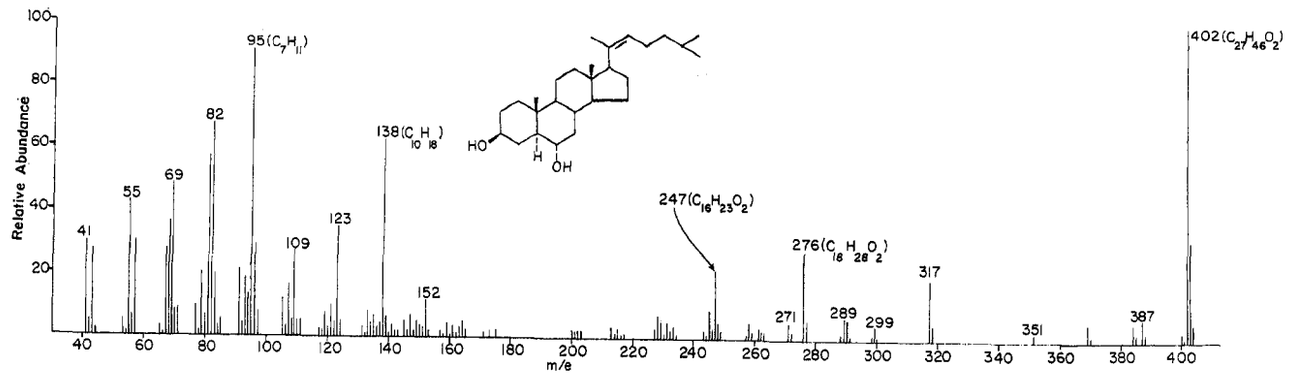


Figure 3.—Mass spectrum (70 eV) of 5α-cholest-20(22)-ene-3β,6α-diol (28).

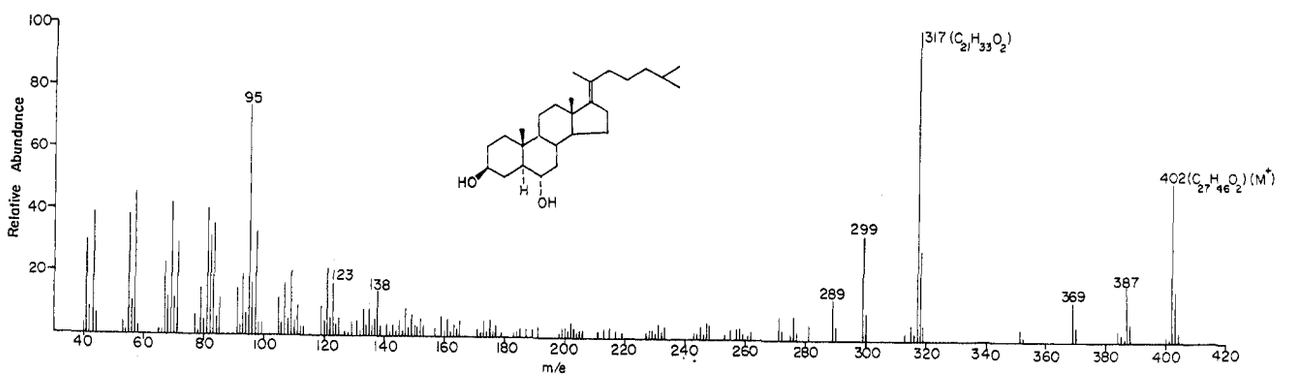
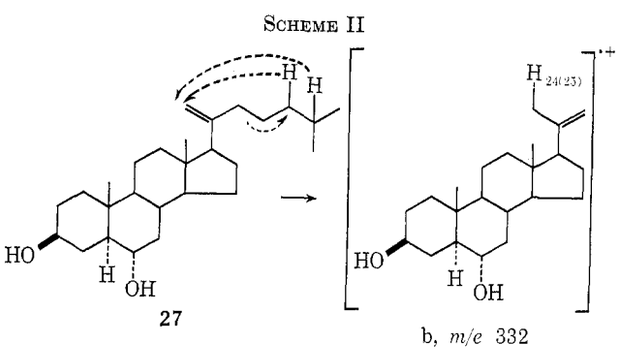
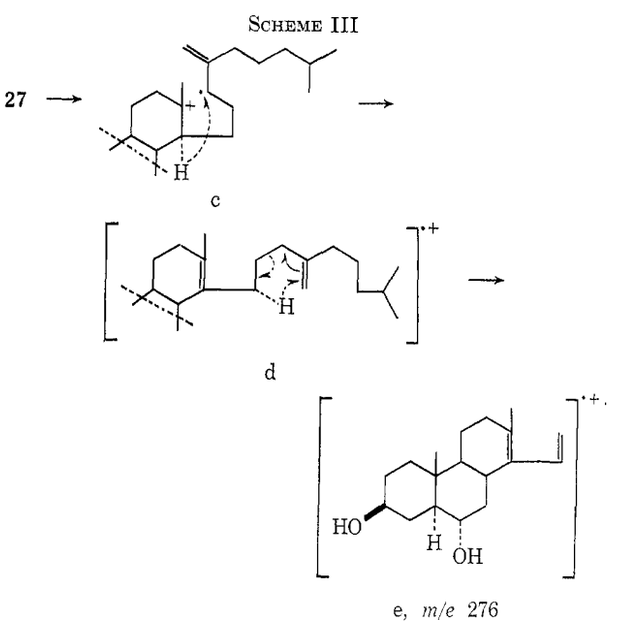


Figure 4.—Mass spectrum (70 eV) of 5α-cholest-17(20)ene-3β,6α-diol (26).

SCHEME II



SCHEME III



Its formation can be explained by invoking ionization of the 13–17 bond to furnish c (tertiary carbonium ion and allylic radical) which after abstraction of the 14α hydrogen[27] could undergo a normal McLafferty rearrangement to furnish ion e (Scheme III). The ion of mass 247 formally corresponds to ring D cleavage[27] but includes the added feature of an unprecedented (among steroids) unidirectional transfer of three hydrogens from rings A, B, or C. The lower mass region is populated by peaks at *m/e* 152, 138, and 95, all of which remain also at low voltage. Possible representations are summarized in Scheme IV and are consistent with the appropriate shifts in the deuterated analogs.

Introduction of an additional double bond in the Δ^{24} position (**30**) radically alters the spectrum (see Figure 2). The molecular ion intensity is reduced while the ion (*m/e* 289) corresponding to loss of the side chain and transfer of two hydrogens[25] (*m/e* 289) becomes an important ion. Labeling work (Table IV) indicated a quantitative transfer of the hydrogen attached to C-17 thus suggesting that its formation is completely analogous to that of other Δ^{24} steroids.[25] In other words the Δ^{24} double bond overshadows the effect of the $\Delta^{20(21)}$ linkage.

The expected cleavage (*m/e* 331) of the doubly, allylically activated 22–23 bond is absent from the spectrum (Figure 2). The ion of mass 273 arises from the molecular ion by loss of the side chain (no transfer of hydrogen) and a mole of water.

By a combination of exact mass measurements (see Figure 2) and deuterium labeling, it could be deter-

(27) L. Tökés, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

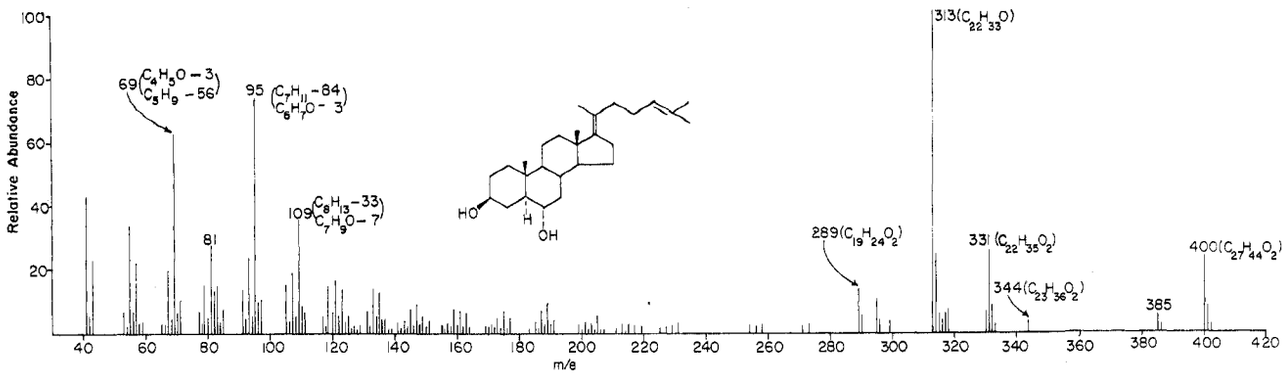


Figure 5.—Mass spectrum (70 eV) of 5α-cholesta-17(20),24-diene-3β,6α-diol (29).

SCHEME IV

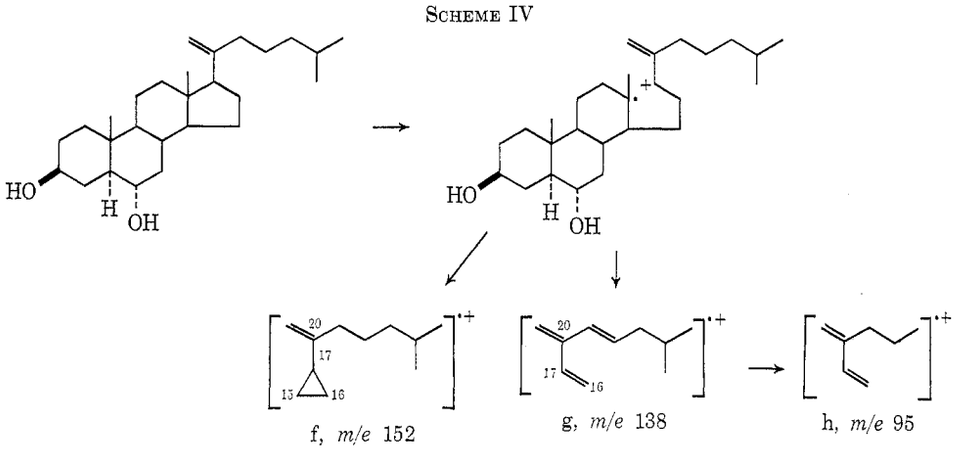


TABLE IV

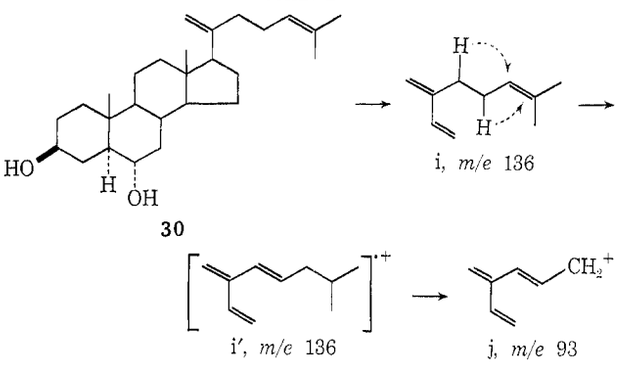
SHIFT IN THE PRINCIPAL PEAKS (*m/e*) IN THE MASS SPECTRA OF DEUTERATED 5α-CHOLESTA-20(21),24(25)-DIENE-3β,6α-DIOL (30)

Compd	M⁺ (%)	*m/e* (%)								
30 (d_0)	400 (30)	290 (41)	289 (71)	273 (30)	271 (23)	109 (65)	107 (42)	93 (100)	81 (60)	69 (89)
30b[a] (17,21,21-d_3)	403 (23)	290 (36)	289 (73)	273 (14)	272 (37)	111 (65)	107 (34)	96 (75)	81 (80)	69 (64)
				274 (17)			108 (19)			
							109 (27)			
30c[a] (26-d_3)	403 (30)	290 (53)	289 (92)	273 (38)	271 (44)	112 (61)	107 (65)	93 (100)	81 (50)	72 (69)
							108 (11)			71 (26)
							109 (27)			

[a] Isotopic purity of **30b** (94% d_3, 5% d_2) and **30c** (88% d_3, 6% d_2, 6% d_1).

mined that the ion of mass 109 consists of the entire side chain, whereas the ion of mass 93 contains carbons 16, 17, 20, 21, 22, and 23 and at least in part is generated by loss of isopropyl (25, 26, 27) from *m/e* 136 (Scheme V).

SCHEME V



$\Delta^{20(22)}$ Olefins (Figure 3).—The mass spectrum of the $\Delta^{20(22)}$ olefin **28** differs from that of the corresponding $\Delta^{20(21)}$ isomer **27** in the more intense fragment of mass 138 which on subsequent loss of isopropyl furnishes *m/e* 95. This parent–daughter relationship (*m/e* 138 → 95) is confirmed by an appropriate metastable peak and the reversal of intensity upon lowering the electron voltage. Deuterium labeling (Table V) indicated that 75% of the ion of mass 95 arises in part (50%) from the reciprocal exchange of the C-17 or C-21 allylic hydrogens with the C-25 hydrogen as depicted in Scheme VI.

$\Delta^{17(20)}$ Olefins (Figure 4).—The mass spectrum of 5α-cholest-17(20)-ene-3β,6α-diol (**26**) is characterized by a strong molecular ion peak and a base peak at *m/e* 317 corresponding to the loss of C_6H_{13}. Formally this represents an *a priori* unfavorable vinylic cleavage as depicted in structure p and labeling with deuterium at positions 21, 22, 23, and 26 confirms this conclusion.

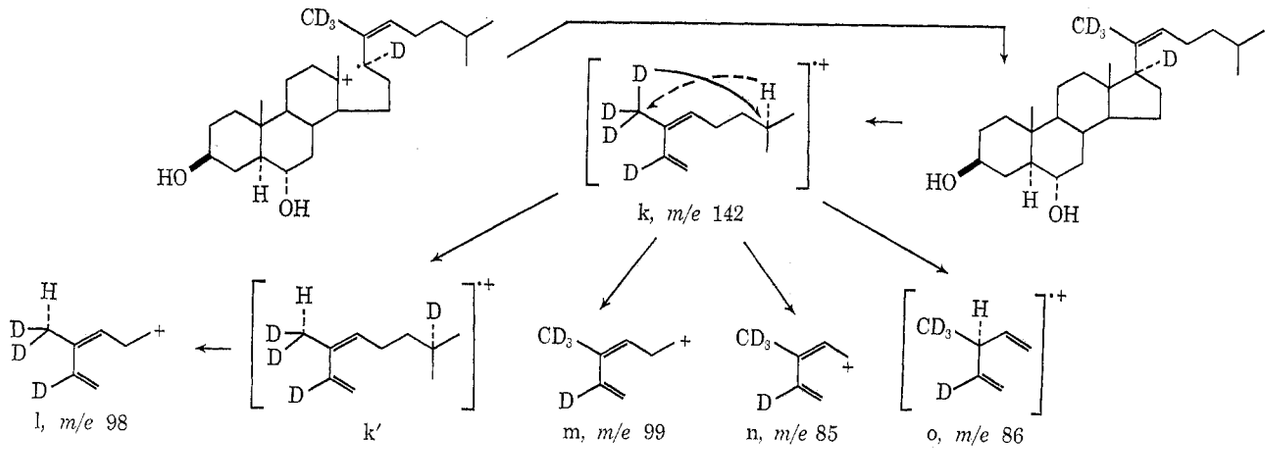
TABLE V

SHIFTS IN THE PRINCIPAL PEAKS (*m/e*) IN THE MASS SPECTRA OF DEUTERATED 5α-CHOLEST-20(22)-ENE-3β,6α-DIOL (**28**)

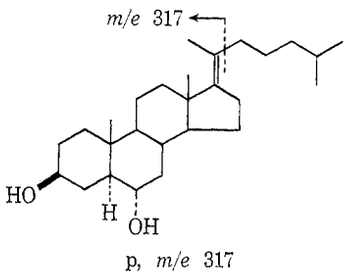
Compd	M⁺ (%)	*m/e* (%)								
28 (d_0)	402 (100)	371 (3)	317 (18)	290 (6) 289 (7)	276 (27)	247 (20)	152 (12)	138 (63)	95 (100)	82 (67) 81 (57)
28b[a] (17,21,21,21-d_4)	406 (100)	373 (4)	320 (10)	290 (8) 289 (9)	276 (37)	247 (31)	156 (10) 155 (7)	142 (67)	99 (99) 98 (71) 95 (57)	86 (72) 85 (46) 82 (45) 87 (30)
28c[a] (22-d_1)	403 (100)		317 (5)	290 (7) 289 (8)	276 (27)	247 (24)	153 (11)	139 (65)	96 (76) 95 (57)	83 (85) 82 (58) 87 (45)
28d[a] (23,23-d_2)	404 (100)	371 (3)	317 (4)	290 (6) 289 (8)	276 (34)	247 (23)	154 (12	140 (49)	97 (89) 95 (55)	84 (46) 83 (51) 82 (26) 87 (34)
28e[a] (24,24-d_2)	404 (100)	371 (7)		290 (10) 289 (12)	276 (38)	247 (30)	154 (15)	140 (82)	97 (64) 96 (72) 95 (100)	83 (73) 82 (70) 87 (66)

[a] Isotopic purity of **28b** (86% d_4, 7% d_3, 7% d_2), **28c** (88% d_2, 12% d_1), **28d** (91% d_2, 4% d_1), and **28e** (94% d_2, 5% d_1).

SCHEME VI



It is conceivable that this fragmentation is triggered by ionization of the 13–14 bond followed by bond formation between C-20 and C-14. Otherwise the spectrum (Figure 4) is characterized by complete absence of ring D cleavage,[27] a minor peak (*m/e* 289) due to loss of side chain and two hydrogens, and the complete absence of any diagnostic ions in the lower mass region.



p, *m/e* 317

Introduction of an additional Δ^{24} double bond (**29**) again alters dramatically the spectrum (see Figure 5). The doubly allylic cleavage (*m/e* 331) which is absent in the spectrum (Figure 2) of the $\Delta^{20(21),24}$-diene **30** becomes an important fragment; subsequent loss of water furnishes the base peak (*m/e* 313). The spectrum is devoid of any ring D cleavage or any other diagnostic ions.

Experimental Section

Melting points (Kofler) are uncorrected. All rotations were determined using chloroform as solvent unless otherwise mentioned. Nmr spectra were recorded ($CDCl_3$ with tetramethylsilane as internal standard) on a Varian T-60 or HA-100 spectrometer. All chemical shifts are reported in δ (parts per million) values. Mass spectra (direct inlet system) were obtained by Mr. R. Ross and Mr. R. T. Conover with an AEI MS-9 or Atlas CH-4 mass spectrometer. All gc was carried out at oven temperatures of 280–285° (unless otherwise specified) using a Hewlett-Packard hp 402 high efficiency gas chromatograph equipped with all-glass U-tube columns packed with 3% OV 25, OV 17 and OV 3 stationary phases coated over Gas-Chrom Q (100–120 mesh). All tlc was performed using E. Merck silica gel Hf_{254}.

5α-Cholestane-3β,6α-diol-23-one (4) and 5α,20α-Cholestane-3β,6α-diol-23-one (5).—Starfish sapogenin[2] diacetate **1b** (50 mg) was dissolved in ethyl acetate (15 ml) and hydrogenated over 10% palladium on charcoal (300 mg) at atmospheric pressure for 50 hr. The mixture was freed of the catalyst and evaporated to furnish a colorless gum which could be fractionated into two components by preparative gc.

4a (retention time 16 min): oil (20%); ir λ_{max} ($CHCl_3$) 1720 cm^{-1} (C=O); CD (MeOH) θ_{290} −2367; nmr δ 0.66, 0.84 (C-18,-C-19 CH_3), 0.86 (d, J = 6.0 Hz, C-26,C-27 CH_3) 2.00 (s, 6 H, 2 $OOCCH_3$), 4.60 (c, 2 H, CHOAc); M^+ 502.

5a (retention time 18 min): oil (80%); ir λ_{max} ($CHCl_3$) 1720 cm^{-1}; CD (MeOH) θ_{290} +1732; nmr δ 0.66, 0.84 (C-18,C-19, CH_3), 0.86 (d, J = 6.0 Hz, C-26,C-27 CH_3), 2.00 (s, 6 H, 2 $OOCCH_3$), 4.60 (c, 2 H, CHOAc); M^+ 502.

Saponification (10% KOH in MeOH) of **4a** furnished **4**: mp 185–187° (plates from MeOH, three crystallizations); λ_{max}

($CHCl_3$) 1700 cm^{-1}; CD (MeOH) θ_{289} −2042; nmr δ 0.66, 0.84 (s, C-18, C-19 CH_3), 0.87 (d, J = 6 Hz, C-26, C-27 CH_3), 3.58 (c, 2 H, CHOH); M^+ 418.34228 (calcd for $C_{27}H_{46}O_3$, 418.34467); mass spectrum m/e (rel intensity) 400 (7, $M^+ - H_2O$), 361 (6%, $M^+ - C_4H_9$), 318 [100, $M^+ - CH_2{=}C(OH)C_4H_9$], 303 (32, 318 − CH_3), 300 (318 − H_2O), 289 (16, M^+ − side chain + 2 H), 285 (21, 303 − H_2O), 231 (7, ring D cleavage + H_2O + 1 H), 213 (11, 231 − H_2O), 161 (14), 141 (21), 127 (37), 122 (58), 95 (57), 85 (57), 81 (43), 57 (53), 43 (36), 41 (35).

Similar saponification of **5a** provided **5**: mp 165–167° (needles from MeOH, three crystallizations); λ_{max} ($CHCl_3$) 1700 cm^{-1}; CD (MeOH) $\theta_{287.5}$ +1693; nmr δ 0.66, 0.83 (s, 6 H, C-18,-C-19 CH_3), 0.87 (d, J = 6 Hz C-26, C-27 CH_3), 3.58 (b, 2 H, 3,6 CHOH); mass spectrum m/e (rel intensity) 418 (M^+, 3), 400 (4, $M^+ - H_2O$), 361 (5), 318 (100), 303 (30), 300 (27), 289, 285, 213, 175 (7), 161 (14), 127 (33), 122 (32), 95 (21), 81 (17), 69 (17), 57 (29), 55 (23), 43 (27), 41 (20).

5α-Cholestane-3β,6α-diol (6) and 5α,20α-Cholestane-3β,6α-diol (7).—Keto diol **4** (10 mg) was heated to 120° with diethylene glycol (0.2 ml) and hydrazine hydrate (0.8 ml) for 5 hr. Potassium hydroxide (0.7 g) was added and the temperature raised to 200° and maintained for 12 hr while a stream of nitrogen was blown over the reaction mixture. Dilution with water, extraction with chloroform, and tlc (chloroform–methanol 9:1, R_f 0.4) followed by crystallization from aqueous methanol furnished **6**, mp 216–218°, M^+ 404, identical in all respects (ir, nmr, mass spectrum) with a synthetic[28] sample of 5α-cholestane-3β,6α-diol.

Similar Wolff–Kishner reduction of **5** furnished **7**: mp 181–182° (methanol); ir λ_{max} (KBr) 3420 cm^{-1}; nmr δ 0.65, 0.82 (s, C-18,C-19 CH_3), 0.88 (d, J = 6.5 Hz, C-26,C-27 CH_3), 3.50 (b, 2 H, 3.6 >CHOH); mass spectrum m/e (rel intensity) 404 (72, M^+), 386 (61, $M^+ - H_2O$), 371 (386 − CH_3), 249 (21, ring D cleavage + 1 H), 231 (59, 249 − H_2O), 213 (21, 231 − H_2O), 141 (26), 123 (32), 109 (36), 95 (100), 87 (47), 69 (46), 57 (52), 55 (69), 43 (100).

Chromium trioxide–pyridine oxidation of **7** furnished the corresponding diketone **9**: mp 125–127° (methanol); ir λ_{max} ($CHCl_3$) 1715 cm^{-1}; nmr δ 0.76, 0.96 (s, C-18,C-19 CH_3), 0.87 (d, J = 6 Hz) (C-26,C-27 CH_3), 1.0–3.0 (c); mass spectrum m/e (rel intensity) 400 (100, M^+) 385 (7, $M^+ - CH_3$), 382 (3, $M^+ - H_2O$), 287 (21, M^+ − side chain), 245 (27, ring D cleavage), 169 (23), 149 (63).

5α-Pregnane-3β,6α-diol-20-one Diacetate.—To a solution of pregnenolone acetate 20-ethylene ketal[19] (6 g) at 0° was added dropwise BH_3–THF (1 M, 40.0 ml) (Alfa Inorganics Ventron). After the mixture stirred at 0° for 30 min, 5% NaOH (125 ml) was carefully added and stirring was continued for an additional 30 min. Hydrogen peroxide (30%, 63 ml) was then added. After 30 min the mixture was poured into ice–water. Chloroform extraction, washing with saturated ferrous sulfate, drying, and acetylation of the residue with acetic anhydride–pyridine for 24 hr afforded, after crystallization from methanol, 4.5 g (60%) of 5α-pregnane-3β,6α-diol-20-one 20-ethylene ketal diacetate: mp 165–167°; $[\alpha]^{21}D$ +31.01° (c 1.138); ir λ_{max} (KBr) 1717–1735 cm^{-1}. A solution of the ketal diacetate (1.2 g) in acetone (50 ml)–water (20 ml) was refluxed with p-toluenesulfonic acid (50 mg) for 5 hr. Dilution with water and ether extraction provided 5α-pregnane-3β,6α-diol-20-one diacetate as colorless gum: ir λ_{max} ($CHCl_3$) 1717–1735 cm^{-1}; nmr δ 0.56, 0.90 (s, C-18,C-19 CH_3), 2.0 (s, 6 H, 2 $OOCCH_3$), 2.03 (s, $COCH_3$), 4.50–5.10 (c, 2 H, 2 CHOAc); M^+ 418.

20-Hydroxy Steroids (12–15).—To a Grignard reagent prepared from isohexyl bromide or 1-bromo-4-methyl-3-pentene (5.0 g) and magnesium (600 mg) was added 800 mg of 5α-pregnane-3β,6α-diol-20-one diacetate or pregnenolone acetate in benzene (25 ml). After removal of ether by distillation, the mixture was refluxed for 15 hr. The complex was hydrolyzed by saturated ammonium chloride and the mixture extracted with chloroform to yield a gummy solid. Preparative tlc over silica gel Hf_{254} (benzene–acetone), crystallization from acetone–hexane, and subsequent multiple crystallizations from aqueous acetone furnished the pure 20α-hydroxy steroids **12–15** (50–60% yield).

Cholest-5-ene-3β,20α-diol[21] (12): mp 120–122°; diacetate **12a** mp 150–151° (lit.[21] mp 123–125 and 155–156°).

Cholesta-5,24-diene-3β,20α-diol (13): mp 96–98° (needles from MeOH, dried over boiling benzene under vacuum); $[\alpha]^{21}D$ −61.1° (c 0.77); mass spectrum m/e (rel intensity) 382 (23, $M^+ - H_2O$), 317 (19, $M^+ - C_6H_{11}$), 297 (28, 317 − H_2O), 271 (90, M^+ − side chain + 2 H + H_2O), 256 (17), 255 (15), 241 (8), 229 (5), 213 (7), 199 (5), 189 (5), 185 (6), 159 (20), 127 [22, $CH_3C(=^+OH)(CH_2)_2CHC(CH_3)_2$], 109 (100, 127 − H_2O), 95 (30), 82 (44), 69 (62), 55 (34), 43 (66).

Acetylation of **13** furnished the monoacetate **13a**, mp 143–145° (needles from hexane).

5α-Cholestane-3β,6α,20α-triol (14): mp 191–193° (aqueous acetone); $[\alpha]^{21}D$ +21.6° (c 0.485); mass spectrum m/e (rel intensity) 402 (32, $M^+ - H_2O$), 384 (402 − H_2O), 369 (6, 384 − CH_3), 335 (91, $M^+ - C_6H_{13}$), 299 (42, 335 − $2H_2O$), 273 (31), 271 (65), 256 (25), 241 (13), 159 (20), 147 (19), 129 [55, $CH_3(=^+OH)(CH_2)_3CH(CH_3)_2$], 95 (90), 81 (50), 69 (100), 55 (77), 43 (51), 41 (36).

Oxidation of **14** with CrO_3/Py furnished **14b**: $[\alpha]^{21}D$ −12.0° (c 0.25); mp 142–144° (needles from aqueous methanol); mass spectrum m/e (rel intensity) 416 (3, M^+), 331 (100, $M^+ - C_6H_{13}$), 313 (60, 331 − H_2O), 288 (46), 287 (35), 138 (57), 129 [69, $CH_3C(=^+OH)(CH_2)_3CH(CH_3)_2$], 111 (48, 129 − H_2O), 69 (50), 55 (33), 43 (32).

5α-Cholest-24-ene-3β,6α,20α-triol (15): mp 162–165° (needles from aqueous acetone); $[\alpha]^{21}D$ +22.6° (c 0.415); mass spectrum m/e (rel intensity) 403 (1), 400 (3), 335 (13), 318 (11), 317 (16), 299 (12), 290 (13), 289 (44), 273 (20), 241 (6), 229 (5), 161 (8), 159 (12), 135 (18), 127 (24), 109 (100), 95 (48), 82 (86), 69 (88), 55 (31), 43 (67).

$\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{21(22)}$ Steroidal Olefins (20–31). A. Dehydration of 20-Hydroxy Steroids (12a–15a) by Phosphorus Oxychloride.—Freshly distilled phosphorus oxychloride (0.4 ml) was added dropwise with vigorous stirring to a solution of 100 mg of the 20-hydroxy steroid (**12a–15a**) in pyridine (4.0 ml). After 12 hr the mixture was poured into ice–water and extracted with ether. Preparative tlc over silica gel HF_{254} (PhH–EtOAc, 9:1) furnished a mixture of olefins which was further fractionated over 15% siliver nitrate impregnated silica gel. The order of elution (benzene) was $\Delta^{17(20)} > \Delta^{20(22)} > \Delta^{20(21)}$ (without Δ^{24}) and $\Delta^{20(22)} > \Delta^{17(20)} > \Delta^{20(21)}$ (with Δ^{24}); percentage yields and gc mobilities of these olefins are presented in Tables I and VI. Both cis and trans $\Delta^{17(20)}$ olefins were produced and separated by preparative gc, whereas only one of the two possible $\Delta^{20(22)}$ olefins could be detected by analytical gc over OV 25, OV 17, and OV 3.

TABLE VI

RETENTION TIMES[a] RELATIVE TO CHOLESTEROL OVER OV 25 AND OV 3 COLUMNS

		OV 25, 3% over Gas-Chrom Q		OV 3, 3% over Gas-Chrom Q	
Compd	Acetate	Parent	Acetate	Parent	Acetate
6	6a			1.84	
7	7a			1.66	
10	10a			1.66	
12	12a		2.13		1.96
13	13a	2.25	2.63	1.75	2.16
14	14a	4.10	4.90	2.90	3.96
15	15a	5.05	6.20	3.27	4.36
20	20a		0.95, 1.10		1.15, 1.23
21	21a	1.00	1.20	0.95	1.26
22	22a	1.10	1.255	0.975	1.35
23	23a	1.20	1.45	1.02	1.30
24	24a	1.20	1.45	1.02	1.38
25	25a		1.60		1.50
26	26a	2.05, 2.20	2.32, 2.55	1.56, 1.75	2.20, 2.38
27	27a	2.30	2.65	1.72	2.39
28	28a	2.55	2.76	1.85	2.56
29	29a	2.70	3.30	1.90	2.31, 2.47
30	30a	2.78	3.35	1.92	2.52
31	31a		3.90		2.75

[a] For gas chromatographic mobilities of 11-functionalized 20α steroid, see J. J. Schneider and L. J. Haffner, *J. Chromatogr.*, **70**, 194 (1972); J. J. Schneider, *Tetrahedron*, **28**, 2717 (1972).

B. Dehydration of 20-Hydroxy Steroids (12a–15a) by Thionyl Chloride.—Thionyl chloride (0.5 ml) was added dropwise to a vigorously stirred ice-cold solution of the 20-hydroxy steroid (100 mg) in pyridine (4.0 ml). After 10 min the mixture was poured into ice–water and worked up as described in A.

(28) S. Wolf, M. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, **24**, 1034 (1959).

C. Dehydration of 14 by Propionic Acid.—The hydroxy steroid **14** (200 mg) was refluxed in propionic acid (5.0 ml) for 12 hr. The mixture was poured into saturated ice-cold sodium carbonate solution and extracted with ether. The ether extract, after saponification with 10% potassium hydroxide in methanol (10 ml) at room temperature for 6 hr, was acetylated to furnish the crude acetate (for details see Table I).

Cholesta-5,20(21)dien-3β-ol (21): mp 111–112°; acetate **21a** mp 101–102° (lit.[15] mp 100–101°); mass spectrum *m/e* (rel intensity) 384 (100, M^+), 369 (9, $M^+ - CH_3$), 366 (9, $M^+ - H_2O$), 351 (23, 369 − H_2O), 314 (6, $M^+ - C_5H_{10}$), 299 (5, 314 − CH_3), 281 (3, 299 − H_2O), 272, 271 (5, 4, M^+ − side chain + 1 H and 2 H), 229 (15, ring D cleavage + 3 H), 213 (13, ring D cleavage + H + H_2O), 159 (7), 152 (4), 145 (12), 138 (3), 133 (8), 95 (31), 81 (25), 69 (27), 55 (36), 43 (68).

Cholesta-5,20(22)-dien-3β-ol (22): mp 135–138° (lit.[3] mp 135–138°); mass spectrum *m/e* (rel intensity) 384 (100%, M^+), 369 (7, $M^+ - CH_3$), 366 (6, $M^+ - H_2O$), 351 (14, 369 − H_2O), 299 (7, $M^+ - C_6H_{13}$), 272, 271 (5, 8, M^+ − side chain + 1 H and 2 H), 258 (10, $M^+ - C_9H_{18}$), 229 (9, ring D cleavage + 3 H), 138 (17, $C_{10}H_{18}$), 95 (35), 81 (23), 69 (19), 57 (17), 55 (20), 43 (15).

Cholesta-5,17(20),24-trien-3β-ol (23): mp 44° (plates from aqueous methanol); $[\alpha]^{21}D$ −64.7° (*c* 0.235); mass spectrum *m/e* (rel intensity) 382 (57, M^+), 367 (9, $M^+ - CH_3$), 313 (95, $M^+ - C_5H_9$), 295 (100, 313 − H_2O), 272, 271 (12, 20, M^+ − side chain + 1 H and 2 H), 187 (21), 159 (31), 149 (45), 145 (28), 135 (32), 133 (37), 121 (31), 119 (40), 109 (55), 107 (42), 105 (37), 95 (88), 93 (37), 91 (39), 81 (52), 69 (70), 55 (46), 41 (70).

Cholesta-5,20(21),24-trien-3β-ol (24): mp 88–91° (needles from aqueous MeOH); $[\alpha]^{21}D$ −55.7° (*c* 0.35); mass spectrum *m/e* (rel intensity) 382 (65, M^+), 367 (23, $M^+ - CH_3$), 364 (11, $M^+ - H_2O$), 349 (23, 367 − H_2O), 272, 271 (49, 100, M^+ − side chain + 1 H and 2 H), 255 (21), 253 (22, 271 − H_2O), 229 (13), 213 (28, ring D cleavage + 1 H + H_2O), 159 (30), 145 (52), 133 (36), 121 (30), 109 (73), 107 (57), 105 (50), 95 (55), 93 (77), 91 (47), 81 (67), 79 (63), 69 (75), 67 (47), 55 (53), 41 (67).

Acetylation of **24** furnished the monoacetate **24a**, mp 75–77° (MeOH).

Cholesta-5,20(22),24-trien-3β-ol acetate (25a): mass spectrum *m/e* (rel intensity) 424 (4, M^+), 364 [100, $M^+ - CH_3C(=O)OH$], 313 (7, M^+ − side chain + 2 H), 282 (67), 254, 253 (32, 43, 364 − side chain + 1 H and 2 H), 228 (19), 213 (21), 211 (17), 199 (8), 185 (6), 173 (7), 159 (15), 158 (18), 157 (15), 147 (21), 145 (29), 143 (18), 136 (27), 135 (29), 133 (24), 121 (38), 119 (23), 117 (11), 109 (45), 107 (37), 105 (36), 95 (33), 93 (55), 91 (27), 82 (21), 81 (36), 80 (25), 79 (26), 69 (30), 67 (26), 58 (17), 55 (30), 43 (52).

5α-Cholest-17(20)ene-3β,6α-diol (26): mp 156–158° (small needles from aqueous MeOH); 98% trans by gc; M^+ 402 (Figure 4).

5α-Cholest-20(21)-ene-3β,6α-diol (27): mp 176–178° (needles from aqueous MeOH); $[\alpha]^{21}D$ +30.4° (*c* 0.31); M^+ 402 (Figure 1).

5α-Cholest-20(22)-ene-3β,6α-diol (28): mp 156–158° (small needles from MeOH–H_2O); $[\alpha]^{21}D$ +23.5° (*c* 0.21); M^+ 402 (Figure 3).

The corresponding diacetate **28a** had M^+ 486. Oxidation of **28** with CrO_3/Py furnished two polymorphic forms of the diketone **28b**: mp 102–103 and mp 124–127° (needles from MeOH); mass spectrum *m/e* (rel intensity) 398 (83, M^+), 383 (12, $M^+ - CH_3$), 328 (8, $M^+ - C_5H_{10}$), 313 (25, $M^+ - C_6H_{13}$), 285 (31, M^+ − side chain + 2 H), 272 (53), 259 (51), 245 (17, ring D cleavage + 1 H), 138 (65, $C_{10}H_{18}$), 123 (35), 109 (45), 95 (100), 81 (65), 69 (61), 55 (60), 41 (46).

5α-Cholesta-17(20),24-diene-3β,6α-diol (29): mp 153–155°; $[\alpha]^{21}D$ +22.17° (*c* 0.212); M^+ 400 (Figure 5). Acetylation furnished the diacetate **29a**, M^+ 484.

5α-Cholesta-20(21),24-diene-3β,6α-diol (30): mp 167–168° (plates from aqueous EtOH); $[\alpha]^{21}D$ +25.0° (*c* 0.30); M^+ 400 (Figure 2). The corresponding diacetate **30a** had mp 108–109° (needles from MeOH); M^+ 484.

5α-Cholesta-20(22),24-diene-3β,6α-diol diacetate (31a): mass spectrum *m/e* (rel intensity) 484 (42 M^+), 424 (14, $M^+ - CH_3CO_2H$), 373 (90, M^+ − side chain + 2 H), 364 (424 − CH_3CO_2H), 342 (15), 315 (7), 314 (25), 313 (16), 283 (22), 255 (32), 253 (14), 227 (17), 213 (17), 161 (35), 136 (74), 135 (75), 133 (62), 121 (60), 110 (70), 109 (90), 107 (58), 95 (58), 93 (88), 82 (89), 81 (64), 80 (53), 69 (60), 67 (43), 55 (53), 43 (100), 41 (49).

5α,20α-Cholestane-3β,6α-diol (7).—5α-Cholest-20(21)-ene-3β,6α-diol (**27**, 25 mg) was dissolved in methanol (7 ml) and hydrogenated over platinum dioxide (80 mg) at atmospheric pressure for 12 hr. The product mixture (gc analysis) was composed of 47% 5α,20α-cholestane-3β,6α-diol (**7**) and 53% 5α-cholestane-3β,6α-diol (**6**). Preparative glc followed by crystallization from aqueous methanol furnished **7**: mp 178–181° (star shaped needles); $[\alpha]^{21}D$ 31.0° (*c* 0.08); undepressed mixture melting point with α diol derived from natural star fish genin **1**. Both diols depicted identical spectral characteristics (ir, nmr, mass spectra). Oxidation of **7** with CrO_3/Py furnished 5α,20α-cholestane-3,6-dione, mp 118–121°, M^+ 400. A similar hydrogenation [10% Pd/C (100 mg), EtOAc (7 ml)] of **27a** and **30a** (25 mg) for 24 hr furnished **6a** and **7a** which on subsequent saponification gave **6** and **7**.

5α,17α,20α-Cholestane-3β,6α-diol (10).—The $\Delta^{17(20)}$ olefinic diacetates **26a** and **29a** (35 mg) were hydrogenated at atmospheric pressure over 10% palladium on charcoal (100 mg) in ethyl acetate (12 ml) for 18 hr. Preparative gc followed by saponification and crystallization furnished **10** (60%), mp 178–180°, which was depressed (mp 175–178°) upon admixture with diol (mp 181–182°) derived from natural starfish genin **1**. Oxidation of **10** with CrO_3/Py furnished 5α,17α,20α-cholestane-3,6-dione (**11**), mp 140–142° (needles from MeOH), M^+ 400.

5α,20α-Cholestane (7b).—Hydrocarbons derived by Wolff–Kishner reduction of the diketone **9** (from natural starfish genin **1**) and from 20α-cholestanol[24] depicted identical mass spectra and gc retention times (OV 25).

1-Bromo-4-methyl-3-pentene-*4*-d_3.—Cyclopropyl methyl ketone (10.0 g, Aldrich Chemical Co.) was mixed with D_2O (50.0 ml). Anhydrous potassium carbonate (200 mg) was added and the mixture stirred at room temperature for 1 week. The mixture, after saturation with sodium chloride, was extracted with ether. Fractionation of the ether residue furnished cyclopropyl-d_3 methyl ketone (4.5 g) which on reaction with a 10-fold excess of methylmagnesium chloride gave cyclopropyl-*methyl*-d_3-methylcarbinol (3.7 g, 70%).

Reaction of the deuterated carbinol with ice-cold hydrobromic acid (35%, 10 ml) for 1 hr furnished 1-bromo-4-methyl-3-penten-*4*-d_3 (cis:trans 1:1) which was used directly in the Grignard reaction.

Deuterated 2-Bromo-4-methylpentanes.—1-Bromo-4-methylpentane-*1,1*-d_2 was prepared by $LiAlD_4$ reduction of isohexanoic acid followed by reaction of the intermediate 1,1-d_2 alcohol with triphenylphosphine and bromine in DMF. 1-Bromo-4-methylpentane-*2,2*-d_2 was synthesized by condensation of isobutyl bromide with diethyl malonate, reaction of the intermediate diethyl isobutylmalonate with NaOD in D_2O and then $POCl_3$, followed by reduction of the intermediate isocaproic acid-*2,2*-d_2 with $LiAlH_4$ with subsequent conversion of the 2-2-d_2 alcohol to the corresponding bromide. Isohexyl bromide-*2,2*-d_2 was prepared by a similar sequence except that the deuterated bromide was employed.

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