(CH_2CH_3), 30.3, 32.9, and 37.8 (C-3, C-4, and CH_2Et), 52.0 (C-6), 55.0 (C-2), 67.8 (C-5); mass spectrum (M^+), calcd for $C_8H_{17}NO$ 143.130995, found 143.130559.

A deuteriochloroform solution containing 7 mg of (±)-pseudoconhydrine was treated with excess anhydrous hydrogen chloride. The resulting milky solution was concentrated, and the crystals were recrystallized from EtOH/EtOAc to give (±)-pseudoconhydrine hydrochloride: mp 157.0–157.5 °C (lit.[2b] mp 135–140 °C); ^1H NMR (200 MHz, D_2O) δ 0.79 (t, J = 7.2 Hz, 3 H, CH_3), 1.1–1.6 (m, 6 H), 1.9–2.1 (m, 2 H), 2.2–2.4 (dd, J = 11.6 and 4.3 Hz, 1 H, C-6 H_e), 2.65 (t, J = 11.6 Hz, 1 H, C-6 H_a), 3.0 (m, 1 H, C-2 methine), 3.8 (m, 1 H, C-5 methine); ^{13}C NMR (20 MHz, D_2O) δ 13.8 (CH_3), 18.9 (CH_2CH_3), 26.2, 30.3, and 34.6 (C-3, C-4, and CH_2Et), 49.3 (C-6), 56.4 (C-2), 63.8 (C-5).

Acknowledgment. We thank The Robert A. Welch Foundation (Grant A-442) for support of this research. The NMR spectrometers used in this research were purchased with the aid of National Science Foundation Grants to Texas A&M University.

Registry No. (±)-1, 5457-27-2; (±)-1·HCl, 41221-92-5; (±)-2, 87830-46-4; (±)-3, 87830-47-5; (±)-4, 87830-31-7; 5, 30503-12-9; (*E*)-5 oxime, 87830-32-8; (*Z*)-5 oxime, 87830-48-6; (±)-6, 87830-33-9; (±)-7a, 87830-45-3; (±)-7b, 87830-34-0; (±)-8a, 87830-36-2; (±)-8b, 87830-40-8; (±)-9·F_3CCO_2 (R = $CH_2CH_2CH_3$; Y = OH), 87830-38-4; (±)-9·F_3CCO_2 (R = CH_3; Y = Cl), 87830-43-1; (±)-10b·Cl, 87830-44-2; (±)-11 (R = $CH_2CH_2CH_3$; Y = OH), 87830-37-3; (±)-11·HBr (R = $CH_2CH_2CH_3$; Y = I), 87830-35-1; (±)-11·HBr (R = CH_3; Y = I), 87830-41-9; (±)-14, 87830-39-5; 4-bromobutene, 5162-44-7; butyraldehyde, 123-72-8; benzyl chloroformate, 501-53-1.

Stereochemistry and Regiochemistry of Electron Impact, Photolytically, and Thermally Induced Eliminations from 5α-Cholestanyl Acetates

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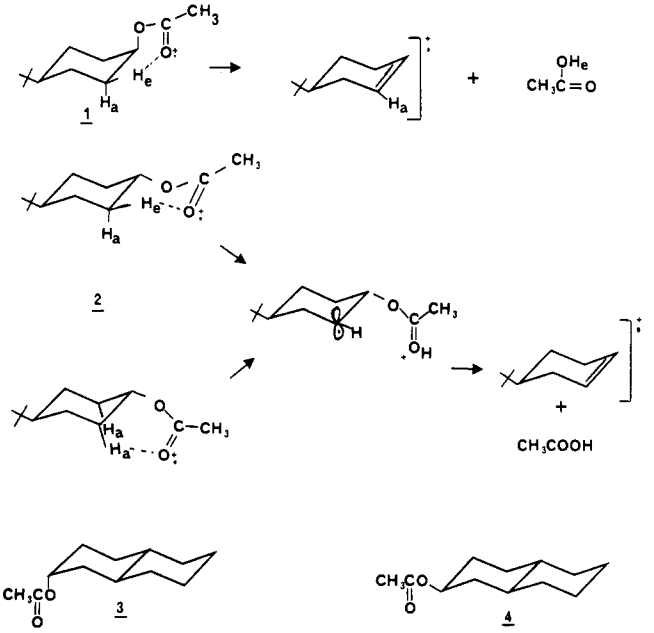
Deuterium-labeled compounds were used to define the stereochemistry and regiochemistry of the electron impact induced eliminations of acetic acid from 5α-cholestanyl 3α-acetate, 4α-acetate, and 6α-acetate. Comparison of the electron impact induced eliminations to the pyrolysis and to the photolysis of the corresponding phenylacetates confirmed that the mass spectral elimination was a stepwise process proceeding through the stable chair conformation of the steroid's cyclohexyl ring. The equatorially oriented 4α- and 6α-acetates fragmented with predominant loss of a secondary trans-equatorial hydrogen, rather than the tertiary cis-axial hydrogen, despite the a priori greater migratory aptitude of tertiary hydrogens. The electron impact induced fragmentation of the 3α-acetate occurred with predominant loss of a C-4 hydrogen; in contrast, the photolysis of the corresponding 3α-phenylacetate results in loss of a C-2 hydrogen. This result can be attributed to the reversibility of the photolytically induced hydrogen-abstraction step.

Steroids are particularly useful as substrates for the investigation of the regiochemistry and stereochemistry of electron impact induced fragmentations. Internuclear distances and angles are well-defined, and the precursors necessary to produce regiospecifically and/or stereospecifically labeled molecules are often readily available. Further, the solution chemistry behaviors and properties of these molecules are comparatively well studied and understood, facilitating the interpretation of results obtained in mass spectrometric studies. Thus, steroid acetates are the focus to the current study on electron impact induced elimination of acetic acid from unsymmetrical derivatives.

It has already been demonstrated that the electron impact induced loss of acetic acid from axially oriented symmetrically substituted cyclohexyl acetates (e.g., *cis*-4-*tert*-butylcyclohexyl acetate, 1, Scheme I) proceeds with predominant loss of a cis-equatorial hydrogen,[1] consistent with hydrogen abstraction from the most stable chair conformer and the requirement for approach of the abstracting oxygen atom within 1.8 Å of the itinerant hydrogen.[2] More surprisingly, it has also been demonstrated that equatorially oriented symmetrically substituted cyclohexyl acetates (e.g., *trans*-4-*tert*-butylcyclohexyl acetate, 2) fragments with predominant loss of a trans-equatorial hydrogen atom.[1] This result is also consistent with hydrogen abstraction from the most stable chair conformer,

Scheme I



provided that the fragmentation is a stepwise process. Then, the unfavorable 1,3-diaxial-like interaction between

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(1) Eadon, G.; Gold, P.; Bacon, E. *J. Am. Chem. Soc.* **1975**, *97*, 5184–5189.

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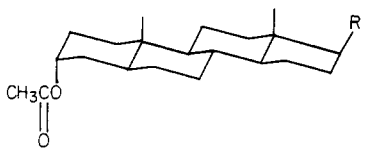
the acetate carbonyl and the C-6 axial hydrogen in the transition state for C-2 axial hydrogen abstraction will lead to preferential abstraction of the C-2 equatorial hydrogen. The latter transition state is nearly strain-free. The proposal that cyclohexyl acetates and related compounds fragment predominantly from their most stable chair conformers in a stepwise process has subsequently been supported in a number of studies.[3,4]

Although the course of this fragmentation in symmetrical cyclohexyl derivatives is now well understood, much less is known about the regiochemistries and stereochemistry of the reaction in unsymmetrical derivatives. For example, a study of the electron impact induced loss of acetic acid from the 2-decalyl acetates **3** and **4** demonstrated stereochemistries consistent with those already described for simple cyclohexyl derivatives and exhibited a strong regiochemical preference for loss of the C-1 hydrogen rather than the loss of a C-3 hydrogen.[5] Further, the regiochemistry of a process thought to be mechanistically similar, the Norrish type II photolysis of the corresponding phenylacetate, was opposite.[5] No convincing explanation could be advanced for this effect. In order to determine its generality and clarify its origin, we studied the stereochemistries and regiochemistries of electron impact and photolytically induced eliminations from 5α-cholestanyl 3α-acetate (**5**).



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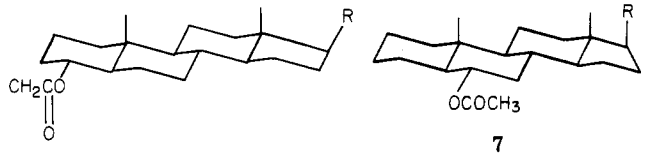


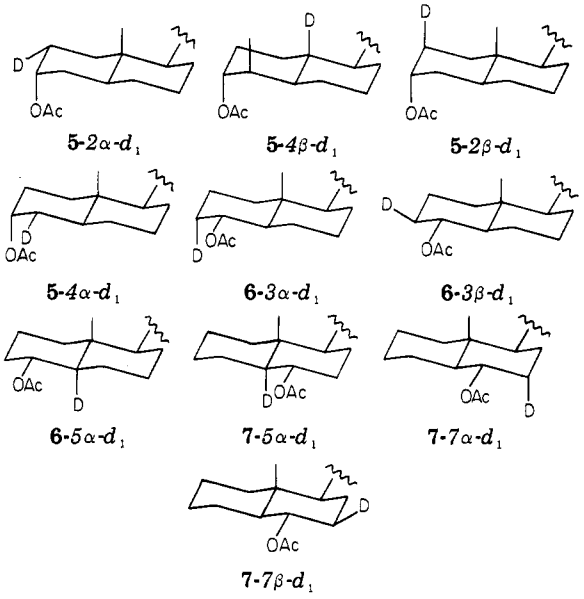
6 **7**

The regiochemistries of and stereochemistries of these eliminations from 5α-cholestanyl 4α-acetate (**6**) and 6α-acetate (**7**) are also studied in this work. These systems are of interest because the aforementioned preference of an equatorial acetate to fragment with predominant loss of an equatorial hydrogen is opposed by the greater migratory aptitude of tertiary hydrogen atoms compared to secondary ones. Thus, these structures will permit qualitative conclusions about the relative importance of these opposing effects in such systems. In addition, they will provide quantitative data necessary to interpret results obtained in ongoing studies of conformationally mobile unsymmetrical systems such as the 2-methylcyclohexyl acetates.

Results

The mass spectra of 5α-cholestanyl 3α-acetate, 4α-acetate, and 6α-acetate exhibit intense peaks at m/z 370 corresponding to the elimination of acetic acid from the molecular ion. At 70 eV, the m/z 370 and 371 ions comprise between 15% and 18% of the total ion current above m/z 40; at threshold, 14 eV, these ions comprise 70–78% of the total ion current above m/z 40. Preparation of the 2,2,4,4-d_4 derivative of the 3α-acetate, the 3,3,5-d_3 derivative of the 4α-acetate, and the 5,7,7-d_3 derivative of the 6α-acetate confirms that acetic acid loss involves predominantly hydrogen abstraction through a six-membered transition state (Table I). Correction of the raw data for isotopic impurities (^{13}C and d_2 and d_3) and extraneous processes (OAc and H_2OAc loss) demonstrates that 72–76% of acetic acid loss involves this process. It should be emphasized that all data were obtained by using a direct insertion probe with the ion source temperature maintained at the minimum necessary for volatilization (95–105 °C). Under these conditions, little pyrolysis of secondary acetates is anticipated; the regiochemistries and stereochemistries observed in these studies are, in fact, inconsistent with thermal reaction.[3,4]

Chart I



A series of monodeuterated cholestanyl 3α-acetates, 4α-acetates, and 6α-acetates was prepared in order to establish the stereochemistry of the electron impact induced hydrogen abstraction step (Chart I). The results obtained are presented in Table I. Although the tabulated data were obtained at 70-eV ionizing voltage, they are in agreement, within experimental error, with data generated at the threshold voltage (nominally 14 eV) for accurate measurement.

The values in the last column of Table I can be used to calculate the "relative rates" of elimination of hydrogen from each labeled position of a particular acetate. For example, for the 3α-acetate **4**, five equations can be written which relate the observed rates of DOAc loss to HOAc loss in each labeled derivative's mass spectrum to the isotope effect I, to the "rate constants" for elimination of a particular hydrogen atom ($k_{2\alpha}$, $k_{2\beta}$, $k_{4\alpha}$, $k_{4\beta}$) and to the rate constant for non-γ-abstraction (k_i). Thus, five equations are generated which can be solved for I, as well as the ratios of various rate constants. Analogous calculations have already been described.[3-5] The isotope effects observed for the 3α-acetate, the 4α-acetate, and the 6α-acetate were, respectively, 1.72 ± 0.19, 1.70 ± 0.24, and 1.35 ± 0.15. Table II contains the relative rate constants calculated for the elimination of each γ-hydrogen atom.

(2) Williams, D. H.; Wilson, J. M.; Budzikiewicz, H.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 2091–2105. Williams, D. H.; Djerassi, C. *Steroids* **1964**, *3*, 259–269. Djerassi, C.; von Mutzenbecher, G.; Fajkos, J.; Williams, D. H.; Budzikiewicz, H. *J. Am. Chem. Soc.* **1965**, *87*, 817–826. Djerassi, C.; Tokes, L. *Ibid.* **1966**, *88*, 536–544. Tokes, L.; LaLonde, R. T.; Djerassi, C. *J. Org. Chem.* **1967**, *32*, 1020–1029.

(3) Eadon, G. *J. Am. Chem. Soc.* **1976**, *98*, 7313–7319. Eadon, G. *Org. Mass Spectrom.* **1977**, *12*, 671–680. Eadon, G.; Jefson, M. *J. Org. Chem.* **1976**, *41*, 3917–3920. Rej, R. N.; Bacon, E.; Eadon, G. *J. Am. Chem. Soc.* **1979**, *101*, 1668–1675.

(4) Eadon, G.; Bacon, E.; Gold, P. *J. Org. Chem.* **1976**, *41*, 171–173.

(5) Rej, R. N.; Taylor, C.; Eadon, G. *J. Org. Chem.* **1980**, *45*, 126–130.

Table I. Electron Impact Induced Elimination of Acetic Acid from Cholestanyl 3α-Acetate, 4α-Acetate, and 6α-Acetate and Labeled Analogues[a]

compd	isotopic purity[b]	$(M^{+\cdot} - OAc)^c$	$(M^{+\cdot} - HOAc)^c$	$(M^{+\cdot} - H_2OAc)^c$ or $(M^{+\cdot} - DOAc)^c$	$\frac{(DOAc\ loss)^{d,e}}{(HOAc\ loss)}$
5α-cholestanyl 3α-acetate (5)		31	100	2	
5α-cholestanyl-2,2,4,4-d_4 3α-acetate	80% d_4	21	79	100	1.50 ± 0.12
5α-cholestanyl-2β-d_1 3α-acetate	98% d_1	33	100	7	0.03 ± 0.007 (0.04 ± 0.007)
5α-cholestanyl-2α-d_1 3α-acetate	97% d_1	47	100	21	0.16 ± 0.02 (0.25 ± 0.02)
5α-cholestanyl-4β-d_1 3α-acetate	98% d_1	32	100	7	0.03 ± 0.007 (0.04 ± 0.007)
5α-cholestanyl-4α-d_1 3α-acetate	97% d_1	28	100	41	0.40 ± 0 (0.65 ± 0)
5α-cholestanyl 4α-acetate (6)		30	100	1	
5α-cholestanyl-3,3,5α-d_3 4α-acetate	83% d_3	21	78.10	100	1.64 ± 0.17
5α-cholestanyl-3β-d_1 4α-acetate	98% d_1	30	100	36	0.36 ± 0.01 (0.56 ± 0.01)
5α-cholestanyl-3α-d_1 4α-acetate	99% d_1	31	100	11	0.09 ± 0.01 (0.12 ± 0.01)
cholestanyl-5α-d_1 4α-acetate	99% d_1	28	100	19	0.17 ± 0.01 (0.24 ± 0.01)
5α-cholestanyl 6α-acetate (7)		29	100	1	
5α-cholestanyl-5α,7,7-d_3 6α-acetate	92% d_3	12	78	100	1.93 ± 0.11
5α-cholestanyl-7β-d_1 6α-acetate	98% d_1	37	100	51	0.54 ± 0.04 (0.71 ± 0.04)
5α-cholestanyl-7α-d_1 6α-acetate	98% d_1	38	100	11	0.09 ± 0.01 (0.12 ± 0.01)
cholestanyl-5α-d_1 6α-acetate	98% d_1	38	100	20	0.18 ± 0.01 (0.24 ± 0.01)

[a] Data were obtained at 70 eV by using a direct insertion probe at a source temperature of 95–105 °C. Peak intensities represent the average of three determinations and are reproducible to ±1 intensity units. [b] Determined from the mass spectrum of the trimethylsilyl ether of the corresponding alcohol. [c] Raw data, averaged over three distinct sets of measurements. [d] Ratios are corrected for isotopic impurities (low d-containing isomers and ^{13}C) and interfering fragmentations ($M^{+\cdot} - OAc$ and $M^{+\cdot} - H_2OAc$). The values in parentheses have been additionally corrected for non C-3 and C-5 abstraction. Error limits include extreme values observed in three distinct sets of measurements. [e] These ratios have been calculated from data obtained near threshold ionization voltages (~14 eV) and agree within experimental error with the above tabulated ratios.

Table II. Relative Rates of Electron Impact Induced Elimination of Hydrogen Atoms from Cholestanyl 3α-Acetate, 4α-Acetate, and 6α-Acetate

compound	hydrogen atom	relative rate of elimination
5α-cholestanyl 3α-acetate	C-2α	0.41 ± 0.03
	C-2β	0.08 ± 0.03
	C-4α	1.0[a]
	C-4β	0.12 ± 0.05
5α-cholestanyl 4α-acetate	C-3α	0.35 ± 0.02
	C-3β	1.0[a]
	C-5α	0.59 ± 0.01
5α-cholestanyl 6α-acetate	C-5α	0.46 ± 0.02
	C-7α	0.27 ± 0.02
	C-7β	1.0[a]

[a] Arbitrarily defined.

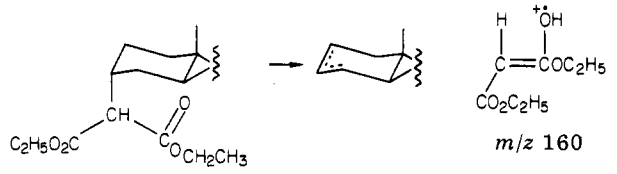
Table III. Comparison of the Regiochemistry of Electron Impact (EI), Photolytically ($h\nu$), and Thermally Induced (Δ) Eliminations from 5α-Cholestanyl 3α-Esters, 4α-Esters, and 6α-Esters

compound	reagent	result
5α-cholestanyl 3α-acetate	EI	C_4/C_2 = 2.28 ± 0.01
5α-cholestanyl 3α-phenylacetate	$h\nu$	0.050 ± 0.006
5α-cholestanyl 3α-acetate	Δ	0.11 ± 0.04
5α-cholestanyl 4α-acetate	EI	C_5/C_3 = 0.44 ± 0.03
5α-cholestanyl 4α-phenylacetate	$h\nu$	0.23 ± 0.07
5α-cholestanyl 4α-acetate	Δ	1.5[a]
5α-cholestanyl 6α-acetate	EI	C_5/C_7 = 0.36 ± 0.03
5α-cholestanyl 6α-phenylacetate	$h\nu$	0.25 ± 0.07
5α-cholestanyl 6α-acetate	Δ	1.61 ± 0.15

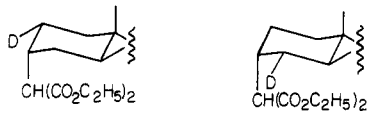
[a] Barton, D. H. R.; Rosenfelder, W. J. *J. Chem. Soc.* 1951, 1048.

In an effort to facilitate interpretation of the mass spectral data, the corresponding phenylacetates were prepared and photolyzed. The regiochemistry of the elimination, as deduced from the isomer content of cholestene produced, appears in Table III. Similarly, the regiochemistry of the pyrolysis of the acetate or corresponding benzoate was determined and is listed in Table III.

Finally, in order to probe the origin of the differing regiochemistries observed in the mechanistically analogous electron impact and photolytically induced elimination from the 3α-acetate and phenylacetate, we examined the mass spectral behavior of the corresponding 3α-diethyl malonate derivative (8). As expected, the unlabeled derivative exhibited a significant peak at m/z 160, corresponding to γ-hydrogen abstraction and elimination of neutral alkene, with charge retention on the oxygen containing fragment. Previous studies[3,5] demonstrated the



8



8-2α-d_1 8-4α-d_1

axial diethyl malonates fragment with virtually clean elimination of cis equatorial γ-hydrogen atoms. Thus, the labeled derivatives 8-2α-d_1 and 8-4α-d_1 were prepared, their mass spectra determined, and the regiochemistry of the electron impact induced elimination calculated, assuming that the reaction was completely specific for equatorial γ-hydrogen abstraction. The relative rate constants calculated correspond to $k_2/k_4 = 0.37$, in good agreement with the corresponding ratio for the 3α-acetate (0.52, Table II).

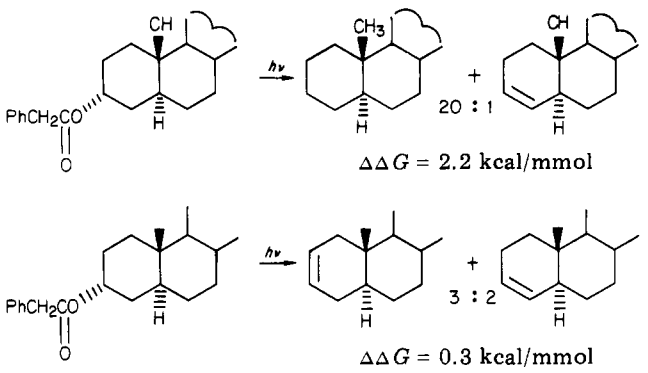
Discussion

The stereochemistry of the electron impact induced elimination from 5α-cholestanyl 3α-acetate parallels that observed for simple cyclohexyl derivatives[3] and for *trans,trans*-2-decalyl acetate.[6] As expected, the fragmentation occurs with predominant loss of the cis equatorial hydrogens from C-2 ($k_{2\alpha}/k_{2\beta} = 5$) and C-4 ($k_{4\alpha}/k_{4\beta} = 8$). The carbonyl oxygen cannot approach the trans β-hydrogens within the requisite 1.8 Å for abstraction if the A ring remains in its chair conformer. In contrast, in the most plausible boatlike conformers of the A ring, the equatorial and axial hydrogens appear comparably accessible. Thus, the strong preference for equatorial hydrogen loss is most consistent with hydrogen abstraction from the stable chair conformer.

The regiochemistry of the electron impact induced elimination is less readily explained. The acetate exhibits a small preference for loss of a C-4, rather than a C-2 hydrogen ($(k_{4\alpha} + k_{4\beta})/(k_{2\alpha} + k_{2\beta}) = 2.3$). Although inspection of models suggests no obvious explanation for the preference, its small size would ordinarily merit little discussion. However, the regiochemistry of the photolysis of the corresponding phenylacetate is sharply opposite; C-2 hydrogen elimination is favored over C-4 hydrogen elimination by about 20:1. Since both processes involve stepwise abstraction of a hydrogen atom through a cyclic six-membered transition state, this dichotomy merits close analysis.

Consideration of the four competing transition states for γ-hydrogen abstraction reveals little reason to expect large preferences for C-2 vs. C-4 hydrogen abstraction in the photolysis of 3. However, the observed preferential formation of the Δ^2-alkene can be rationalized. Very substantial evidence exists that the Norrish type II photolysis proceeds with reversible γ-hydrogen abstraction.[7] If so, the distribution of products from an unsymmetrical phenylacetate will be dependent on the rates of the final step, cleavage of the C–O single bond. These rates will be influenced by the stability of the alkene product. This proposal is supported by an earlier report that photolysis of the corresponding 2-decalyl phenylacetate also forms an excess of the more stable alkene but with a small preference[6] (Scheme II); the smaller preference is consistent with the smaller difference between the stabilities of the alkene isomers.[8]

Scheme II



The electron impact induced process, on the other hand, exhibits a modest preference in the opposite direction; C-4 hydrogen loss is favored over C-2 hydrogen loss. Two explanations can be advanced for this result. First, the relative instabilities of the ionized alkenes produced in the mass spectral process may be very different from those of the corresponding neutral alkenes. The origins of the stability difference between Δ^2 and Δ^3 trans-fused steroids are subtle. Calculations demonstrated that the effect arises because of a more unfavorable interaction between the C-6 β-hydrogen atom and the C-19 methyl group in the Δ^3-alkene and because of greater angle strain produced when one of the common atoms in two fused cyclohexyl rings is allylic.[8] Both effects may be attenuated when the structures involved are *ionized* rather than neutral alkenes. Evidence suggests that ionized ethylene prefer a twisted geometry.[9] Thus, the failure of the electron impact induced process to proceed predominantly with loss of the C-2 hydrogen may be due to the fact that the stabilities of the ionized Δ^2- and Δ^3-alkene products are similar or reversed.

Alternatively, this discrepancy could be rationalized by postulating that the electron impact induced hydrogen transfer is irreversible; some evidence has been advanced supporting this proposal for McLafferty-type rearrangements in other classes of compounds.[10] Green's recent PEPICO-based studies of 2-butyl acetate support this conclusion strongly.[11] If the γ-hydrogen abstraction is irreversible, and if the C-2 and C-4 radicals each give a high yield of ionized alkene, the observed regiochemistry will be determined primarily by the relative rates of C-2 vs. C-4 hydrogen abstraction. As already discussed, those rates should be similar.

For differentiation between these hypothesis, a derivative was sought that would undergo fragmentation in a manner mechanistically similar to the acetates but that would generate a neutral alkene product. The corresponding diethyl malonate derivatives have been demonstrated to undergo such a fragmentation with stereo-

(6) Eadon, G.; Alonso, C.; Valente, H. *J. Org. Chem.* **1983**, *48*, 520–526.
(7) Yarchak, M. L.; Dalton, J. C.; Saunders, W. H., Jr. *J. Am. Chem. Soc.* **1973**, *95*, 5224–5227; **1973**, *95*, 5228–5232. Wagner, P. J.; Zepp, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 287–289. Wagner, P. J. *Ibid.* **1967**, *89*, 5898–5901.
(8) Turner, R. B.; Meador, W. R.; Winkler, R. E. *J. Am. Chem. Soc.* **1957**, *79*, 4122–4126. Corey, E. J.; Sneer, R. A. *Ibid.* **1955**, *77*, 2505–2509.
(9) Lorquet, A. J.; Lorquet, J. C. *J. Chem. Phys.* **1968**, *49*, 4955–4961. Mulliken, R. S. *Tetrahedron* **1959**, *5*, 253–274.
(10) Djerassi, C.; Dieckman, J. *J. Org. Chem.* **1967**, *32*, 1005–1012.
(11) Green, M. M.; McCluskey, R. J.; Vogt, J. *J. Am. Chem. Soc.* **1982**, *104*, 2262–2269.

chemistry similar to that of the acetates.[3,4] The diethyl 3α-malonate derivative of cholestane and its 2α-d_1 and 4α-d_1 analogues were prepared and their mass spectra examined; a clear preference for elimination of a C-4 hydrogen was observed. On the basis of studies with other cyclohexyl-based derivatives, the elimination reaction should proceed with virtually complete abstraction of a C-2α or C-4α hydrogen.[3,4] That assumption permits calculation of k_{C-2}/k_{C-4} as 0.37, in agreement with the corresponding ratio for the 3α-acetate (0.52). Thus, the differing regiochemistries observed in the photolytic and electron impact induced eliminations cannot be attributed to differences in the geometries of the alkene products.

The most plausible explanation for the insensitivity of the regiochemistry of the mass spectral reaction to product stability postulates that the direction of elimination is determined only by the relative rates of the initial hydrogen abstraction reaction; hydrogen abstraction rates will not be influenced by the same factors as those influencing the stability of Δ^2- and Δ^3-alkenes.

Recognition of the mechanistic dichotomy between the photochemical and electron impact induced fragmentation permits explanation of another curious observation; the stereospecificity of the electron impact induced loss of acetic acid from *trans*-4-*tert*-butylcyclohexyl acetate (**2**) is significantly more biased toward equatorial hydrogen abstraction (k_{eq}/k_{ax} = 3.8) than the photolytically induced loss of phenylacetic acid from the corresponding phenylacetate (equatorial loss/axial loss = 1.8). The net stereochemistry of the mass spectral process is determined solely by the initial hydrogen abstraction step. For the reversible photochemical process, the apparent net stereochemistry will be influenced by the rate at which each intermediate biradical cleaves to form product. Since an equatorially oriented sp^3 orbital at C-2 is oriented trans to the C–O bond at C-1, distortion of these bonds toward coplanarity as necessary in the transition state for alkene formation must be an unfavorable process. Thus, rehybridization/inversion will precede cleavage. On the other hand, the C-2 orbital produced by axial hydrogen abstraction is oriented cis relative to the C-1 C–O bond and cleavage should be facile. The slower fragmenting radical resulting from equatorial hydrogen abstraction will more often react by reverse hydrogen transfer to regenerate starting phenylacetate, and thus less net elimination of that hydrogen will be observed. It is noteable that the earlier observation that the stereochemistry of the starting material is not scrambled during photolysis[4] supports the hypothesis that back hydrogen transfer is faster than radical–radical interconversion, as required in this argument.

The electron impact induced eliminations from 5α-cholestanyl 4α- and 6α-acetates (**6** and **7**) proceed with predominant loss of a secondary equatorial hydrogen rather than a tertiary axial hydrogen (for **6**, $k_{3\beta}/k_{5\alpha}$ = 1.7; for **7**, $k_{7\beta}/k_{5\alpha}$ = 2.2). The rigid steroid structure limits the multiplicity of boat-like conformers attainable by these acetates; inspection of models demonstrates that in all accessible boat-like conformers, axial hydrogens are more readily approached by the abstracting carbonyl group than the equatorial hydrogen. Thus, this result is consistent with earlier studies indicating that the hydrogen abstraction step occurs predominantly from cyclohexyl groups in their stable chair conformers. Further, it demonstrates that the fairly subtle steric interactions in the competing transition states for hydrogen abstraction outweigh the well-established preference for tertiary rather than secondary hydrogen abstraction. This result is in excellent quantitative agreement with those reported earlier for *trans,trans*-1-decalyl acetate ($k_{2\alpha}/k_{8a\beta}$ = 2.2) in a study of a simpler but analogous system.

An analogous result was obtained after photolysis of the phenylacetates corresponding to **6** and **7**; elimination of a secondary hydrogen atom to form the less stable alkene predominated over tertiary hydrogen atom elimination to form the less stable alkene by about four to one. This result, as already discussed, is produced by the interplay of various factors including the rates of transfer of various hydrogens, the rates of back-transfer reactions, and the rates of cleavage of the intermediate radicals. It is, however, noteable that the reaction provides a potentially useful and predictable synthetic pathway leading from an alcohol to the corresponding anti-Markovnikov alkene that can be accomplished without the use of strongly basic or acidic reagents or elevated temperatures. The utility of this transformation is presently limited by the modest yields obtained (ca. 50%); however, no efforts have been made to optimize reaction conditions.

The pyrolysis of acetates **4** and **6** proceeds predominantly with elimination of the C-5α hydrogen to generate the more substituted alkene. The pyrolytic elimination has already been demonstrated to proceed with clean cis stereochemistry, as required by its concerted nature. Thus, the competing paths involve abstraction of a secondary axial hydrogen vs. a tertiary axial hydrogen; stereochemical effects are thus negligible, and the predominant factor is the stability of the isomeric alkene products. However, the dichotomy between the regiochemistry of the pyrolytic reaction and the electron impact induced process confirms that the latter is mechanistically destined and is entirely consistent with the proposed nonconcerted nature of the latter process.

Conclusions

Earlier studies on the stereochemistries of thermal, photolytic, and electron impact induced eliminations from symmetrically substituted cyclohexyl acetates and phenylacetates led to the conclusion that the electron impact induced process was nonconcerted and exhibited an unusual net trans stereochemistry when the acetate was equatorially oriented. The present study of the regiochemistry and stereochemistry of these processes in steroidal 3α, 4α, and 6α derivative have confirmed these conclusions. In addition, it has provided clear evidence for a mechanistic distinction between the electron impact and photolytic eliminations. Experimental data demonstrated that this result could not be ascribed to the different products produced in the two reactions (a neutral vs. an ionized alkene) and led to the postulate that the hydrogen abstraction step is irreversible in the mass spectral process; the reversibility of the photolytic hydrogen transfer is well-known. The net anti-Markovnikov elimination exhibited in the photolysis of the 4α- and 6α-phenylacetates suggests that the reaction may, with further optimization, have potential as a mild, predictable synthetic route from alcohols to certain poorly accessible alkenes.

Experimental Section

Melting points are uncorrected. All mass spectra were run by using the direct insertion probe at 70 eV on an AEI MS 902 spectrometer, with the exception of the mass spectra of diethyl 3α-cholestanylmalonate and deuterated analogues which were run on an AEI MS 30 spectrometer (on line with a Data General DS-55 data system). All reactions were run under nitrogen. Thin-layer chromatography of the alcohols employed aluminum oxide HF-254 (type 60/E) 0.75-mm-thick slides. The isotropic purity of each

labeled alcohol was determined from the mass spectrum of the corresponding trimethylsilyl ether derivative.[10] Acetates were prepared by the acetic anhydride–pyridine procedure,[12] they were purified by thin-layer chromatography (silica gel HF-254, 0.75-mm-thick slides) prior to mass spectral analysis.

All hygroscopic solvents were dried and distilled under a nitrogen atmosphere prior to use. Diethyl ether and tetrahydrofuran were distilled from lithium aluminum hydride. Diglyme was dried over calcium hydride overnight and distilled from lithium aluminum hydride at reduced pressure. Ethylamine was purchased as a 70% solution in water and distilled twice, the last time from potassium hydroxide.

Δ^2-Cholestene was prepared according to the procedure by Malunowicz, Fajkos, and Sorm.[12] Column chromatography on silica gel, eluting with petroleum ether, afforded pure alkene; mp 72–73 °C (lit.[12] mp 74–75 °C); NMR and mass spectra were in agreement with the proposed structure.

Δ^3-Cholestene was prepared by a modification of the Caglioti reaction developed by Cambie, Rutledge, Scott and Woodgate.[13] The resulting brown oil was purified by column chromatography on silica gel; petroleum ether used as eluent; mp 68–69 °C (lit.[12] mp 72–73 °C). NMR and mass spectra were in agreement with the proposed structure.

Δ^4-Cholestene was prepared according to the procedure of Hallsworth, Henbest, and Wrigley.[14] Column chromatography on 20% silver nitrate impregnated silica gel,[15] eluting with 6.5% benzene–hexane, gave the desired alkene; mp 82–84 °C (lit.[14] mp 83–84 °C). NMR and mass spectra were in agreement with the proposed structure.

Δ^5-Cholestene was prepared by an adaptation of the procedure by Gassman and Marshall.[16] A solution of 3.6 g of sodium, 5.3 mL of *tert*-butyl alcohol, and 42 mL of dry tetrahydrofuran was brought to reflux, treated with cholesteryl chloride (4.05 g, 10 mmol), and stirred under reflux for 24 h. The solution was carefully decanted over 55 g of ice and extracted with ether, and the ether phase was washed with saturated sodium chloride and water. Purification by column chromatography over silica gel, eluting with petroleum ether, yielded the alkene; mp 89–90 °C (lit.[17] mp 95 °C). NMR and mass spectra were in agreement with the proposed structure.

Δ^6-Cholestene was prepared by reduction of 5α-cholestanyl 6-tosylhydrazone with *n*-butyllithium according to the procedure by Herz et al.[18] Column chromatography on 20% silver nitrate impregnated silica gel[15] yielded pure alkene; mp 82–84 °C (lit.[18] mp 87 °C). NMR and mass spectrum were in agreement with the proposed structure.

Δ^3-Cholestene-*3*-d_1 was prepared from Δ^4-cholesten-3α-*d*-3β-ol[19] in an analogous manner to that described for Δ^3-cholestene (vide supra). Column chromatography of the brown oil on silica gel, eluting with petroleum ether, afforded the deuterated alkene. Melting point and TLC were identical with the unlabeled alkene. The mass spectrum exhibited a molecular ion at m/z 371.

Δ^6-Cholestene-*7*-d_1. Δ^5-Cholesten-7α-d_1-7β-ol[20] was reacted in the same manner as that described for Δ^3-cholestene. Purification by column chromatography, eluting with petroleum ether, yielded Δ^6-cholestene-7-d_1. Melting point and TLC were identical with that of Δ^6-cholestene. The mass spectrum exhibited a molecular ion at m/z 371.

Cholestan-*3,3,5*α-*d*$_3$-4β-ol. Cholestan-3,3,5α-d_3-4-one[21] (0.358 g, 0.92 mmol), lithium aluminum hydride (0.114 g, 3.0 mmol), and 8 mL dry tetrahydrofuran were refluxed for 2 h. The reaction was cooled to room temperature and cautiously decomposed with 15 mL of 5% acetic acid. The solution was extracted with ether and washed with 10% sodium bicarbonate and water. Evaporation of the solvent followed by column chromatography on silica gel, eluting with benzene, afforded 0.258 g of cholestan-3,3,5α-d_3 (0.73 mmol, 79% yield), mp 130–131 °C (lit.[21] mp 132 °C).

Δ^4-Cholestene-*3,3*-d_2. A solution of cholestan-3,3,5α-d_3-4β-ol (0.258 g, 0.73 mmol) in 3.7 mL of pyridine was treated while cooling in an ice bath with 0.3 mL of phosphorous oxychloride following a procedure previously described.[22] Melting point and TLC were identical with that of the unlabeled alkene. The mass spectrum exhibited a molecular ion at m/z 372.

Cholestan-*5*α,*7,7*-*d*$_3$-6β-ol. Cholestan-5α,7,7-d_3-6-one[23] (0.460 g, 1.18 mmol) was reduced with lithium aluminum hydride in a strictly analogous fashion to that described for cholestan-3,3,5α-d_3-4β-ol. Obtained was 0.325 g of cholestan-5α,7,7-d_3-6β-ol (0.8 mmol, 69% yield); mp 79–81 °C (lit.[17] mp 81 °C).

Δ^5-Cholestene-*7,7*-d_2. Cholestan-5α,7,7-d_3-6β-ol (0.325 g, 0.8 mmol) was treated with phosphorus oxychloride in exactly the same manner as that described for Δ^4-cholestene-3,3-d_2. Melting point was identical with that of Δ^5-cholestene. The mass spectrum exhibited a molecular ion at m/z 372.

5α-Cholestan-3α-ol. Cholestan-3-one was reduced with lithium tri-*sec*-butylborohydride as described previously.[24] The alcohol was purified by thin-layer chromatography, eluting with a 9:1 mixture of benzene:ether; mp 181–182 °C (lit.[11] mp 187–188 °C). NMR spectrum was in agreement with that reported in the literature.[25]

5α-Cholestan-*2,2,4,4*-*d*$_4$-3α-ol. Cholestan-2,2,4,4-d_4-3-one[21] was reduced with lithium tri-*sec*-butylborohydride as described previously.[24] The alcohol was purified by thin-layer chromatography. Melting point and physical properties were identical with the unlabeled alcohol. The trimethylsilyl ether derivative gave a molecular ion at m/z 464 ($C_{30}H_{52}D_4OSi$).

5α-Cholestan-*2*β-*d*$_1$-3α-ol was prepared by treating 2α,3α-oxido-5α-cholestane[26] with lithium aluminum deuteride as described previously.[12] Preparative thin-layer chromatography generated pure alcohol. The alcohol had physical properties and NMR spectrum identical with those of the corresponding unlabeled compound. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-*2*α-*d*$_1$-3α-ol was prepared by deuteroboration of Δ^2-cholestene as described previously.[27] Preparative thin-layer chromatography afforded the pure alcohol. Physical properties and NMR spectrum were identical with that of unlabeled alcohol. The trimethylsilyl ether derivative had a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-*4*β-*d*$_1$-3α-ol was prepared by treatment of 3α,4α-oxido-5α-cholestane[28] with lithium aluminum deuteride as described previously.[12] Pure alcohol was obtained after preparative thin-layer chromatography. Physical properties and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative had a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

(12) Malunowicz, I.; Fajkos, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1960**, *25*, 1359–1370.
(13) Cambie, R. C.; Rutledge, P. S.; Scott, D. W.; Woodgate, P. D. *Aust. J. Chem.* **1979**, *32*, 695–698.
(14) Hallsworth, A. S.; Henbest, H. B.; Wrigley, T. I. *J. Chem. Soc.* **1957**, 1969–1974.
(15) Breslow, R.; Baldwin, S.; Flechtner, T.; Kalicky, P.; Liu, S.; Washburn, W. *J. Am. Chem. Soc.* **1973**, *95*, 3251–3262.
(16) Gassman, P. G.; Marshall, J. L.; Yates, P., Eds. *Org. Synth.* **1968**, *48*, 68–72.
(17) Fieser, L. F.; Fieser, M. "Steroids"; Reinhold: New York, 1967; p 253.
(18) Herz, J. E.; Gonzalez, E.; Mandel, B. *Aust. J. Chem.* **1970**, *23*, 857–859.
(19) Prepared by reduction of cholest-4-en-3-one with lithium aluminum deuteride: McKennis, H., Jr.; Gaffney, G. W. *J. Biol. Chem.* **1948**, *175*, 217–220.
(20) Δ^5-Cholesten-7-one was prepared by allylic oxidation of Δ^5-cholestene with *tert*-butyl chromate: Heusler, K.; Wellstein, A. *Helv. Chim. Acta* **1952**, *35*, 284–294. The enone was reduced with lithium aluminum deuteride according to ref 19.
(21) Prepared by deuterium exchange of 5α-cholestan-4-one with Na–CH_3OD–D_2O: Williams, D. H.; Wilson, J. M.; Budzikiewicz, H.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 2091–2105.
(22) Bernstein, S.; Rittel, R.; Williams, J. H.; *J. Am. Chem. Soc.* **1953**, *75*, 4830–4832. Ikegawa, S.; Obatake, N.; Hosada, H.; Nambara, T. *Chem. Pharm. Bull.* **1978**, *26*, 3450–3456.
(23) Prepared by deuterium exchange of 5α-cholestan-6-one with Na–CH_3OD–D_2O, see ref 21.
(24) Brown, H. C.; Krishnamurthy, S. *J. Am. Chem. Soc.* **1972**, *94*, 7159–7161.
(25) Sadler 9981.
(26) 2α,3α-Oxidocholestane was prepared by oxidation of Δ^2-cholestene with *m*-chloroperbenzoic acid, according to ref 12.
(27) Sondheimer, F.; Nussim, M. *J. Am. Chem. Soc.* **1961**, *95*, 630–631. Ikegawa, S.; Obatake, N.; Hosoda, H.; Nambara, T. *Chem. Pharm. Bull.* **1978**, *26*, 3450–3456.
(28) Prepared by oxidation of Δ^3-cholestene with *m*-chloroperbenzoic acid, according to ref 11.

5α-Cholestan-4α-d_1-3α-ol was prepared by deuteroboration of Δ^3-cholestene as described previously.[27] Preparative thin-layer chromatography gave pure alcohol. Physical properties and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative gave a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-4α-ol was prepared by hydroboration of Δ^4-cholestene as described previously.[27] The alcohol was purified by preparative thin-layer chromatography; mp 181–183 °C (lit.[27] mp 188–189 °C).

Cholestan-3,3,5α-d_3-4α-ol was prepared by deuteroboration of 3,3,-d_2-Δ^4-cholestene as described previously.[22] Preparative thin-layer chromatography afforded pure alcohol. Physical properties and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 463 ($C_{30}H_{53}D_3OSi$).

5α-Cholestan-3β-d_1-4α-ol was prepared by hydroboration of Δ^3-cholestene-3-d_1 by the same procedure used for the hydroboration of Δ^4-cholestene (vide supra).[27] Thin-layer chromatography afforded pure alcohol. Melting point, TLC, and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-3α-d_1-4α-ol was prepared by deuteroboration of Δ^3-cholestene by using the same procedure described for the deuteroboration of Δ^2-cholestene (vide supra).[27] Purification by thin-layer chromatography gave pure alcohol. Melting point, TLC, and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

Cholestan-5α-d_1-4α-ol was prepared by deuteroboration of Δ^4-cholestene.[27] Purification by thin-layer chromatography afforded the labeled alcohol. Melting point, TLC, and NMR spectrum were identical with that of 5α-cholestan-4α-ol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-6α-ol was prepared by hydroboration of Δ^5-cholestene.[27] Recrystallization from acetone yielded pure alcohol. NMR and mass spectra are in agreement with the proposed structure; mp 125–126 °C (lit.[29] mp 128–129 °C).

Cholestan-5α,7,7-d_3-6α-ol was prepared by deuteroboration of Δ^5-cholestene-7,7-d_2. Preparative thin-layer chromatography afforded pure alcohol. Physical properties and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative gave a molecular ion at m/z 463 ($C_{30}H_{53}D_3OSi$).

5α-Cholestan-7β-d_1-6α-ol was prepared by hydroboration of Δ^6-cholestene-7-d_1.[27] Preparative thin-layer chromatography gave the desired alcohol. Melting point, TLC, and NMR were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

5α-Cholestan-7α-d_1-6α-ol was prepared by deuteroboration of Δ^6-cholestene.[26] Purified by preparative thin-layer chromatography. Melting point, TLC, and NMR spectrum were identical with the unlabeled alcohol. The trimethylsilyl ether derivative gave a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

Cholestan-5α-d_1-6α-ol was prepared by deuteroboration of Δ^5-cholestene[27] and purified by thin-layer chromatography. Melting point, TLC, and NMR spectrum were identical with that of the unlabeled alcohol. The trimethylsilyl ether derivative exhibited a molecular ion at m/z 461 ($C_{30}H_{55}DOSi$).

Diethyl 3α-cholestanylmalonate was prepared from 5α-cholestanyl 3β-tosylate[12] following the procedure by Shoppee and Stephenson.[30] Purification by column chromatography, eluting with petroleum ether, afforded the diethyl malonate derivative; mp 108–110 °C. NMR and mass spectrum were in agreement with the proposed structure.

5α-Cholestan-2α-d_1-3β-ol was prepared from 5α-cholestan-2α-d_1-3α-ol by an oxidation–reduction sequence (Jones, 20 °C, 15 min;[31] lithium aluminum hydride in THF[19,27]). Thin-layer chromatography and NMR spectrum showed the desired alcohol to be the major product (>99%). The mass spectrum showed a molecular ion at m/z 389.

5α-Cholestanyl-2α-d_1 3β-tosylate was prepared by 5α-cholestan-2α-d_1-3β-ol with *p*-toluenesulfonyl chloride in pyridine.[12] The product was recrystallized from petroleum ether. Thin-layer chromatography, melting point, and NMR spectrum were identical with that of 5α-cholestanyl 3β-tosylate.

Diethyl 3α-cholestanyl-2α-d_1-malonate was prepared from 5α-cholestanyl-2α-d_1 3β-tosylate by using the same procedure described for diethyl 3α-cholestanylmalonate[30] (vide supra) and then purified by column chromatography, eluting with petroleum ether. Melting point, TLC, and NMR spectrum were similar to that of diethyl 3α-cholestanylmalonate. Mass spectrum exhibited a molecular ion at m/z 531.

5α-Cholestan-4α-d_1-3β-ol was prepared from 5α-cholestan-4α-d_1-3α-ol by an oxidation–reduction sequence (Jones, 20 °C, 15 min;[31] lithium aluminum hydride in THF[19,27]). Thin-layer chromatography and NMR spectrum showed the desired alcohol to be the major product (>98%); the mass spectrum exhibited a molecular ion at m/z 389.

5α-Cholestanyl-4α-d_1 3β-tosylate was prepared by treating 5α-cholestan-4α-d_1-3β-ol with *p*-toluenesulfonyl chloride in pyridine.[12] Thin-layer chromatography, melting point, and NMR spectrum were identical with that of 5α-cholestanyl 3β-tosylate.

Diethyl 3α-cholestanyl-4α-d_1-malonate was prepared from 5α-cholestanyl-4α-d_1 3β-tosylate by using the same procedure described for diethyl 3α-cholestanylmalonate[30] (vide supra) and purified by column chromatography, eluting with petroleum ether. Melting point, TLC, NMR spectrum were similar to that of diethyl 3α-cholestanylmalonate. The mass spectrum exhibited a molecular ion at m/z 531.

Photolysis of 5α-Cholestanyl 4α-Phenylacetate, 5α-Cholestanyl 6α-Phenylacetate, and 5α-Cholestanyl 3α-Phenylacetate. The phenylacetates were prepared from the corresponding alcohols, following the procedure of Kaiser and Woodruff.[32] Photolyses were conducted according to the general procedures of Yarchak, Dalton, and Sanders.[33] The phenylacetates were irradiated as thoroughly degassed 0.02 M solutions in hexane by using eight Rayonet 2537-Å lamps. The isomer distribution of the alkenes were determined by gas chromatography on a Varian 3700 instrument equipped with a 6-ft 3% SP-2250 glass column at 230 °C. The alkenes exhibited chemical shifts and retention times identical with those of authentic samples. The relative proportions of the alkenes were unchanged (within experimental error) as the amount of starting material consumed was varied from 5% to 50%.

Pyrolysis of 5α-Cholestanyl 6α-Acetate and 5α-Cholestanyl 3α-Acetate. A 30-mg sample of the acetate was placed in a sublimation tube equipped with dry ice–acetone-cooled cold finger. The sublimation apparatus was flushed with nitrogen and placed in a 63.5:36.5 mol % potassium nitrate:sodium thiocyanate bath[34] (preheated to 330 °C) for 20 min. The regiochemistry of the elimination was determined by gas chromatography employing the same conditions used in the photolyses.

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Registry No. **5**, 1107-59-1; **5**-ol, 516-95-0; **5** (phenylacetate), 42921-35-7; **5**-2,2,4,4-d_4, 87697-32-3; **5**-ol 2,2,4,4-d_4, 5618-07-5; **5**-2,2,4,4-d_4 Me_3Si ether, 87697-57-2; **5**-2β-*d*, 87697-33-4; **5**-ol-2β-*d*, 87697-58-3; **5**-2β-*d* Me_3Si ether, 87697-59-4; **5**-2α-*d*, 87697-34-5; **5**-ol-2α-*d*, 87697-60-7; **5**-2α-*d* Me_3Si ether, 87697-61-8; **5**-4β-*d*, 87697-35-6; **5**-ol-4β-*d*, 87697-62-9; **5**-4β-*d* Me_3Si ether, 87697-63-0; **5**-4α-*d*, 87697-36-7; **5**-ol-4α-*d*, 87697-64-1; **5**-4α-*d* Me_3Si ether, 87697-65-2; **6**, 7755-25-1; **6**-ol, 19586-33-5; **6** (phenylacetate),

(29) Bull, J. R.; Jones, E. R. H.; Meakins, G. D. *J. Chem. Soc.* **1965**, 2601–2626.
(30) Shoppee, C. W.; Stephenson, R. J. *J. Chem. Soc.* **1954**, 2230–2242.
(31) Djerassi, C.; Engle, R. R.; Bowers, A. *J. Org. Chem.* **1956**, *21*, 1547–1549.
(32) Kaiser, E. M.; Woodruff, R. A. *J. Org. Chem.* **1970**, *35*, 1198–1199.
(33) Yarchak, M. L.; Dalton, J. C.; Saunders, W. H., Jr. *J. Am. Chem. Soc.* **1973**, *95*, 5224–5232.
(34) Gordon, A. J.; Ford, R. A. "The Chemist's Companion: A Handbook of Practical Data, Techniques and References"; Wiley: New York, 1972; p 42.

87697-48-1; 6-3,3,5α-d_3, 87697-37-8; 6-ol-3,3,5α-d_3, 87697-66-3; 6-3,3,5α-d_3 Me_3Si ether, 87697-67-4; 6-3β-d, 87697-38-9; 6-ol-3β-d, 87697-68-5; 6-3β-d Me_3Si ether, 87697-69-6; 6-3α-d, 87697-39-0; 6-ol-3α-d, 87697-70-9; 6-3α-d Me_3Si ether, 87697-71-0; 6-5α-d, 87697-40-3; 6-ol-5α-d, 87697-72-1; 6-5α-d Me_3Si ether, 87697-73-2; 7, 54657-02-2; 7-ol, 19043-45-9; 7 (phenylacetate), 87697-49-2; 7-5α,7,7-d_3, 87697-41-4; 7-ol-5α,7,7-d_3, 87697-74-3; 7-5α,7,7-d_3 Me_3Si ether, 87697-75-4; 7-7β-d, 87697-42-5; 7-ol-7β-d, 87697-76-5; 7-7β-d Me_3Si ether, 87697-77-6; 7-7α-d, 87697-43-6; 7-ol-7α-d, 87697-78-7; 7-7α-d Me_3Si ether, 87697-79-8; 7-5α-d, 87697-44-7; 7-ol-5α-d, 74051-94-8; 7-5α-d Me_3Si ether, 87697-80-1; 8, 87697-45-8; 8-2α-d, 87697-46-9; 8-4α-d, 87697-47-0; 5α-Δ²-cholestene, 570-73-0; 5α-Δ³-cholestene, 28338-69-4; Δ⁴-cholestene, 16732-86-8; Δ⁵-cholestene, 570-74-1; cholesteryl chloride, 910-31-6; 5α-cholestanyl-6-tosylhydrazone, 18069-88-0; 5α-Δ⁶-cholestene, 28338-70-7; 5α-Δ³-cholestene-3-d, 59582-32-0; Δ⁴-cholesten-3β-ol-3α-d, 1251-63-4; 5α-Δ⁶-cholestene-7-d, 87697-50-5; Δ⁵-cholesten-7β-ol-7α-d, 87697-51-6; cholestan-4β-ol-3,3,5α-d_3, 87697-53-8; cholestan-4-one-3,3,5α-d_3, 87697-52-7; Δ⁴-cholestene-3,3-d_2, 87697-54-9; cholestan-6β-ol-5α,7,7-d_3, 87697-55-0; cholestan-6-one-5α,7,7-d_3, 4321-23-7; Δ⁵-cholestene-7,7-d_3, 87697-56-1; 5α-cholestan-3-one, 566-88-1; 5α-cholestan-3-one-2,2,4,4-d_4, 13976-58-4; 2α,3α-oxido-5α-cholestane, 1753-61-3; 3α,4α-oxido-5α-cholestane, 1249-56-5; 5α-cholestan-β-ol-2α-d, 87697-81-2; 5α-cholestanyl-2α-d 3β-tosylate, 87711-05-5; 5α-cholestan-3β-ol-4α-d, 87697-82-3; 5α-cholestanyl-4α-d 3β-tosylate, 87697-83-4; cholest-4-en-3-one, 601-57-0; Δ⁵-cholesten-7-one, 22033-90-5; 5α-cholestan-4-one, 566-51-8; 5α-cholestan-6-one, 570-46-7.

Synthesis of Ribooligonucleotides Using the 4-Methoxybenzyl Group as a New Protecting Group for the 2'-Hydroxyl Group[1]

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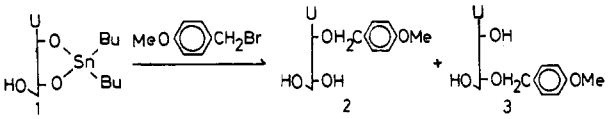
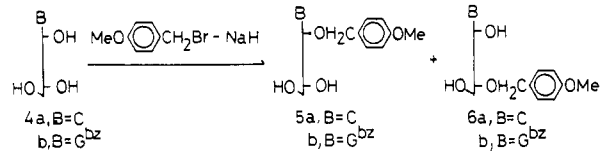
The 4-methoxybenzyl group was introduced to protect the 2'-hydroxyl group of uridine, cytidine, and N^2-benzoylguanosine by treatment of 2',3'-O-(dibutylstannylene)uridine or NaH-treated nucleosides with 4-methoxybenzyl bromide. The 2'-O-(4-methoxybenzyl)nucleosides can be used as useful starting materials for the synthesis of 3',5'-linked ribooligonucleotides. The 4-methoxybenzyl group was removed rapidly from the ribooligonucleotides by treatment with triphenylmethyl fluoroborate, and the completely deblocked ribooligonucleotides were characterized by enzymatic hydrolysis.

Tetrahydropyranyl[2] and methoxytetrahydropyranyl[3] groups have commonly been used to protect the 2'-hydroxyl group of ribonucleotsides in the synthesis of ribooligonucleotides. We have also reported[4] that the tetrahydropyranyl group can be used for synthesis of the 3'- and 5'-reiterated terminal sequences of Rous sarcoma virus 35S RNA. These protecting groups were introduced onto the 2'-hydroxyl group of ribonucleotides through 3',5'-protected ribonucleosides as intermediates.[2-5] Consequently, direct protection of the 2'-hydroxyl group of ribonucleosides has been a crucial problem in the chemical synthesis of ribooligonucleotides. However, only few examples of the direct protection of the 2'-hydroxyl group of ribonucleosides can be found in the literature.[6]

Scheme I



Scheme II



Recently, we found[7] that the 4-methoxybenzyl group can be introduced directly to protect the 2'-hydroxyl group of adenosine with 4-methoxybenzyl bromide and can again be removed rapidly by treatment with triphenylmethyl fluoroborate.[8] The 2'-protected adenosine has been shown to be a useful starting material for the synthesis of ribooligonucleotides.

In this paper, we report the synthesis of 2'-O-(4-methoxybenzyl)uridine, cytidine, and N^2-benzoylguanosine by using 4-methoxybenzyl bromide[9] and their employment in the synthesis of ribooligonucleotides.

Synthesis of 2'-O-(4-Methoxybenzyl)uridine (2). Moffatt and his co-workers have recently reported[6b] that

(1) This manuscript represents part 21 in a series on oligonucleotide synthesis. For the previous paper this series, see: Takaku, H.; Kamaike, K.; Suetake, M. *Chem. Lett.* **1983**, 111.

(2) Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2315. van Boom, J. H.; Owen, G. R.; Preston, J.; Ravindrsnathan, T.; Reese, C. B. *J. Chem. Soc.* **1971**, 3230. Werstiuk, E. S.; Neilson, T. *Can. J. Chem.* **1972**, *50*, 1283. Neilson, T.; Wastrodowski, E. V.; Werstiuk, E. S. *Ibid.* **1973**, *51*, 1068. Gregorire, R. J.; Neilson, T. *Ibid.* **1978**, *56*, 487.

(3) Reese, C. B.; Saffhill, R.; Sulston, J. E. *Tetrahedron* **1970**, *26*, 1023; *J. Am. Chem. Soc.* **1967**, *89*, 3366.

(4) Takaku, H.; Nomoto, T.; Kamaike, K. *Nucleic Acids Res., Symp. Ser.* **1980**, *No. 8*, s91.

(5) Markiewicz, W. T. *J. Chem. Res., Synop.* **1979**, 24; *J. Chem. Res. Miniprint* **1979**, 0.81. Markiewicz, W. T.; Wiewioroski, M. *Nucleic Acids Res., Spec. Publ.* **1978**, *No. 4*, 185. Honda, S.; Terada, K.; Sato, Y.; Sekine, M.; Hata, T. *Chem. Lett.* **1982**, 15.

(6) (a) Kikugawa, K.; Sato, F.; Tsuruo, T.; Imura, N.; Ukita, T. *Chem. Pharm. Bull.* **1968**, *16*, 1110. (b) Wagner, D.; Verheyden, J. H. P.; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 24. (c) Christensen, L. F.; Broom, A. D. *Ibid.* **1972**, *37*, 3389. (d) Ohtsuka, E.; Tanaka, S.; Ikehara, M. *Nucleic Acids Res.* **1974**, *1*, 1351. (e) *Chem. Pharm. Bull.* **1977**, *25*, 949. (f) *Synthesis* **1977**, 453. (g) Ohtsuka, E.; Wakabayashi, T.; Tanaka, S.; Tanaka, T.; Ohie, K.; Hasegawa, A.; Ikehara, M. *Chem. Pharm. Bull.* **1981**, *29*, 318.

(7) Takaku, H.; Kamaike, K.; Tsuchiya, M. *Nucleic Acids Res., Symp. Ser.* **1981**, *No. 10*, 171. Takaku, H.; Kamaike, K. *Chem. Lett.* **1982**, 189.

(8) Dauben, H. J.; Honnen, J. L. R.; Harmon, K. M. *J. Org. Chem.* **1960**, *25*, 1442.

(9) Kornblum, N.; Smiley, R. A.; Backwood, R. T.; Iffland, D. C. *J. Am. Chem. Soc.* **1955**, *77*, 6269.

0022-3263/84/1949-0051$01.50/0