(5) There appears to be a certain sequence of events including *Tetradymia*, time, water, and possibly another plant which leads to the bighead syndrome.
(6) There may be some danger in characterizing a sheep toxin with mice, but symptoms and morphological slides were identical.
(7) When expanded this resonance is a quartet with J = 1.1 Hz. All nmr values are in δ (parts per million).
(8) G. A. Eagle, D. E. A. Rivett, D. H. Williams, and R. G. Wilson, *Tetrahedron*, **25**, 5227 (1969).
(9) H. Ishii, T. Tozyo, and H. Minato, *Tetrahedron*, **21**, 2605 (1965).
(10) L. H. Zalkow, J. W. Ellis, and Sister M. Roger Brennen, *J. Org. Chem.*, **28**, 1705 (1963).
(11) L. Novotny, Z. Samek, J. Harmatha, and F. Šorm, *Collect. Czech. Chem. Commun.*, **34**, 1739 (1969).
(12) (a) A. Stoll, R. Morf, A. Rheiner, and J. Renz, *Experentia*, **12**, 360 (1956); (b) see ref 8.
(13) T. Kubota in "Cyclopentanoid Terpene Derivatives," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, New York, N. Y., 1969, pp 279–356.
(14) P. V. Demarco, E. Farkas, D. Doddrell, B. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, **90**, 5480 (1968).
(15) R. E. Rondeau and R. E. Sievers, *J. Amer. Chem. Soc.*, **93**, 1522 (1971).
(16) Since these quartets, especially the narrower one, are AB quartets, each doublet is not strictly one proton.

Sterol Metabolism. XXXII. Radiation-Induced Oxidation of Isomeric Cholesten-3β-ols[1]

Martin J. Kulig[2] and Leland L. Smith*

Division of Biochemistry, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550

Received May 20, 1974

The air oxidation induced by ^{60}Co γ radiation of cholest-4-en-3β-ol, 5α-cholest-6-en-3β-ol, and 5α-cholest-7-en-3β-ol yielded allylic hydroperoxides and other oxidized derivatives. The Δ^4-sterol gave cholest-4-en-3-one, 6β-hydroperoxycholest-4-en-3-one, 3β-hydroxycholest-4-ene 6α-hydroperoxide, and cholest-4-ene-3β,6α-diol. The Δ^6-sterol gave cholesterol 7α- and 7β-hydroperoxides, the epimeric cholest-5-ene-3β,7-diols, 3β-hydroxycholest-5-en-7-one, and 5α-cholest-6-ene-3β,5-diol but no 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide. The Δ^7-sterol gave the epimeric 3β-hydroxy-5α-cholest-7-ene 6-hydroperoxides, the epimeric 5α-cholest-7-ene-3β,6-diols, 3β-hydroxy-5α-cholest-7-en-6-one, and cholesta-5,7-dien-3β-ol. Pyrolysis of either Δ^7-6-hydroperoxide gave the corresponding 5α-cholest-7-ene-3β,6-diol, 3β-hydroxy-5α-cholest-7-en-6-one, and cholesta-5,7-dien-3β-ol. Reaction pathways for oxidations by radiation-induced processes of the isomeric Δ^4-, Δ^5-, Δ^6-, and Δ^7-sterols and for their photosensitized oxidations in which singlet molecular oxygen is implicated were compared.

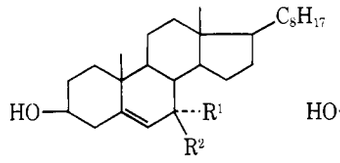
We have recently demonstrated that radiation-induced oxidation of the Δ^5-sterol cholesterol (**1a**) by air afforded the epimeric 7-hydroperoxides **1b** and **1c**,[3] with the quasi-equatorial[4] 7β-hydroperoxide **1c** predominating. In contrast oxidation of cholesterol by excited-stage (singlet) molecular oxygen yielded the 5α-hydroperoxide **3b** as major product, with small amounts of the epimeric 3β-hydroxycholest-4-ene 6-hydroperoxides **2b** and **2c** but with neither 7-hydroperoxide **1b** nor **1c** formed.[5] This distinction in major products formed provides a means of differentiation between participation of ground-state or of singlet molecular oxygen in chemical and enzymic[6] reactions.

Although the mechanism of attack of singlet molecular oxygen on steroid olefins has been extensively studied, free-radical oxidations by ground-state molecular oxygen have not received systematic attention. In order to determine whether additional distinctions between free-radical and singlet molecular oxygen oxidations of sterol olefins existed, as well as to provide additional substrates for use as probes in reactions in which cholesterol was unsuited, we examined the oxidation of cholest-4-en-3β-ol (**2a**), 5α-cholest-6-en-3β-ol (**3a**), and 5α-cholest-7-en-3β-ol (**4a**) induced by γ radiation of ^{60}Co for comparison with their previously reported behavior toward singlet molecular oxygen.

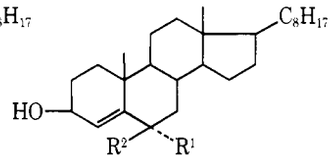
Oxidation of the Δ^4-3β-alcohol **2a** yielded cholest-4-en-3-one (**5a**) as major product, with 6β-hydroperoxycholest-4-en-3-one (**5b**) as the major hydroperoxide product. Small amounts of the 6α-hydroperoxide **2b** were also formed. The Δ^4-3-ketone **5a** was stable to ^{60}Co γ radiation, but irradiation of the pure Δ^4-6β-hydroperoxide **2c** yielded **5b** along with previously recognized thermal decomposition products cholest-4-ene-3β,6β-diol (**2e**) and 3β-hydroxycholest-4-en-6-one (**6**).[5] Accordingly, the 6β-hydroperoxide **5b** did not derive from **5a** but must have derived from **2c**. Inadequate amounts of the 6α-hydroperoxide **2b** precluded study of its radiation stability.

Formation of the 6-ketone **6** as a thermal decomposition product from **2c** was previously supported by detection of its pyrolysis products cholest-4-ene-3,6-dione (**7**) and 5α-cholestane-3,6-dione (**8**) among pyrolysis products from **2c**.[5] Direct observation of **6** following irradiation of **2c** now clearly establishes this reaction pathway of the 6β-hydroperoxide **2c**. However, pyrolysis of the 6β-hydroperoxide **5b** also gave the 3,6-diketones **7** and **8** as prominent products, a point previously suggested but not examined.[5] Derivation from **5b** of the saturated 3,6-diketone **8** must involve intermediate formation of 6β-hydroxycholest-4-en-3-one (**5c**) which then rearranges to **8**. Formation from **2c** of the Δ^4-3,6-diketone **7** may occur by three pathways— **2c** to **5b** to **7**, **2c** to **5b** to **5c** to **7**, or **2c** to **6** to **7**—whereas that of the saturated 3,6-diketone **8** may be by two pathways—**2c** to **5b** to **5c** to **8** and **2c** to **6** to **8**.

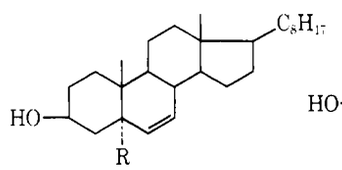
Oxidation of the Δ^6-3β-alcohol **3a** gave unexpectedly the epimeric cholesterol 7-hydroperoxides **1b** and **1c** as major products, the 7β-hydroperoxide **1c** predominating. The secondary oxidation products cholest-5-ene-3β,7α-diol (**1d**), cholest-5-ene-3β,7β-diol (**1e**), 5α-cholest-6-ene-3β,5-diol (**3c**), and 3β-hydroxycholest-5-en-7-one (**9**) were also formed. However, no 5α-hydroperoxide **3b** was detected. The 5α-hydroperoxide **3b** was fairly stable to ^{60}Co γ radiation in air, with less than 10% being converted to a mixture of **1b**, **1c**, **1d**, **1e**, and **3c**. Accordingly, were **3b** formed from **3a** initially, **3b** would have survived and been detected. Thus, initial formation of **3b** with complete allylic rearrangement to **1b**, epimerizatior of **1b**, and thermal decomposition of **1b**, **1c**, and **3b** cannot account for the presence of **1b**, **1c**, **1d**, **1e**, and **3c** as products from **3a**. Residual parent sterol **3a** recovered after ^{60}Co irradiation was not contaminated with detectable amounts of cholesterol; so the product 7-hydroperoxides **1b** and **1c** did not derive by initial isomerization of the Δ^6-double bond to the Δ^5 position, followed by oxidation of cholesterol thereby formed. Rath-



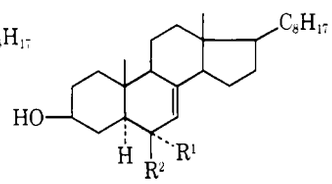
1a, $R^1 = R^2 = H$
b, $R^1 = OOH$; $R^2 = H$
c, $R^1 = H$; $R^2 = OOH$
d, $R^1 = OH$; $R^2 = H$
e, $R^1 = H$; $R^2 = OH$



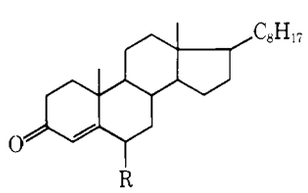
2a, $R^1 = R^2 = H$
b, $R^1 = OOH$; $R^2 = H$
c, $R^1 = H$; $R^2 = OOH$
d, $R^1 = OH$; $R^2 = H$
e, $R^1 = H$; $R^2 = OH$



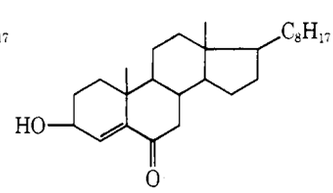
3a, R = H
b, R = OOH
c, R = OH



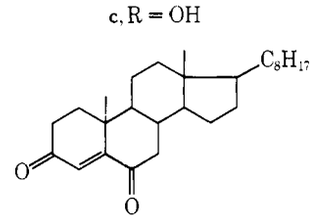
4a, $R^1 = R^2 = H$
b, $R^1 = OOH$; $R^2 = H$
c, $R^1 = H$; $R^2 = OOH$
d, $R^1 = OH$; $R^2 = H$
e, $R^1 = H$; $R^2 = OH$



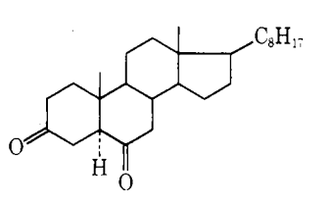
5a, R = H
b, R = OOH
c, R = OH



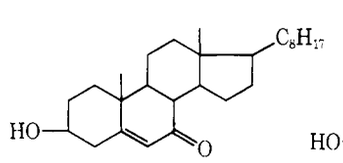
6



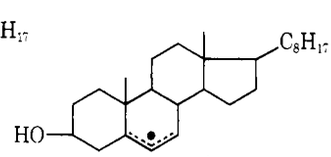
7



8



9

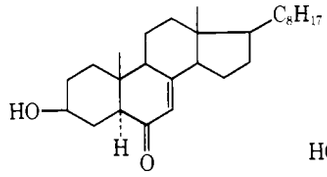


10

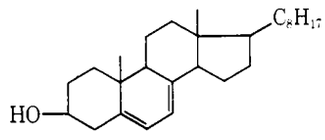
er, initial generation from **3a** of a mesomeric free-radical species **10** from which the Δ^5-7-hydroperoxides **1b** and **1c** were derived appears to be the case. The quasiequatorial 7β-hydroperoxide **1c** predominated, as it does in the radiation-induced oxidation of **1a**.[3] The secondary products **1d, 1e,** and **9** are clearly accounted for as thermal decomposition products of the initially formed 7-hydroperoxides **1b** and **1c**.[3,4,7] The 3β,5α-diol **3c** must derive by allylic rearrangement of the Δ^5-7α-alcohol **1d** in that the parent 5α-hydroperoxide **3b** was not implicated.

Oxidation of the Δ^7-sterol **4a** yielded a complex mixture of products including four peroxides, two of which were prominent and which could be recovered. The major hydroperoxide was 3β-hydroxy-5α-cholest-7-ene 6β-hydroperoxide (**4c**); the other was the epimeric 6α-hydroperoxide (**4b**). The 6β-hydroperoxide **4c** was also found in commercial samples of **4a** which had been stored for some time in the laboratory.[8] Identity of the Δ^7-6-hydroperoxides was established by sodium borohydride reduction to the corresponding alcohols, thus **4b** to **4d** and **4c** to **4e.**

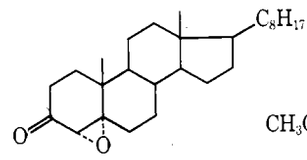
Other products formed from **4a** were the epimeric Δ^7-3β,6-diols **4d** and **4e,** the Δ^7-6-ketone **11,** and cholesta-5,7-dien-3β-ol (**12**), all recognized as thermal decomposition



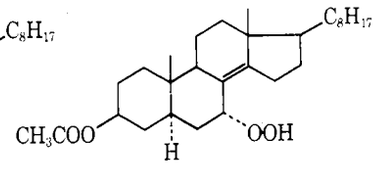
11



12



13



14

products of the parent 6-hydroperoxides **4b** and **4c.** Pyrolysis of the 6α-hydroperoxide **4b** gave the corresponding 6α-alcohol **4d,** the 6-ketone **11,** and the 5,7-diene **12** as major product. Pyrolysis of the epimeric 6β-hydroperoxide **4c** gave likewise the 6β-alcohol **4e,** the 6-ketone **11,** and the 5,7-diene **12,** also as major product.

The Δ^7-3β,6-diols **4d** and **4e** both survived pyrolysis in part, but both were dehydrated to the 5,7-diene **12** as chief product. The 3β,6β-diol **4e** additionally was epimerized to **4d,** dehydrogenated to the 6-ketone **11,** and dehydrated to a nonpolar derivative, presumably cholesta-2,4,6-triene or cholesta-3,5,7-triene. Thermal epimerization of the 6β-alcohol **4e** but not of the epimeric 6α-alcohol **4b** taken with the greater number of pyrolysis products from **4e** infers the less stable quasiaxial conformation for the 6β-hydroxy group. However, in distinction to the Δ^5-7α-oxygenated sterols **1b** and **1d**,[4,7a] neither the Δ^7-6-alcohols **4d** and **4e** nor the Δ^7-6-hydroperoxides **4b** and **4c** were epimerized in acetone solutions.

The 5,7-diene **12** detected as the major pyrolysis product from **4b, 4c, 4d,** and **4e** was present as a minor component among the oxidation products from **4a.** The instability of **12** in air is notorious, and irradiation of **12** yielded a very complex mixture of oxidized products including sterol peroxides, some of which had chromatographic properties similar to uncharacterized sterol peroxide derivatives derived by irradiation of **4a.** The two unidentified peroxides from **4a** may include the theoretically possible 9α- or 14α-hydroperoxides of **4a** or the Δ^6-5α,8α-epidioxide derivative of the 5,7-diene **12.**

The facile thermal elimination reactions of **4b, 4c, 4d,** and **4e** yielding the 5,7-diene **12** provoke recollection of the conversion of the Δ^7-sterol **4a** to **12** by liver microsomal enzymes, which conversion requires molecular oxygen.[9] An enzyme–molecular oxygen complex has been suggested[10] which moderates cis elimination of the 5α- and 6α-hydrogens,[11] the 6α-hydrogen being removed as a proton and not as hydride ion.[12] Although the Δ^7-6-alcohols **4d** and **4e** are converted by liver microsomal enzymes to cholesterol, presumably *via* the 5,7-diene **12,** they do not appear to be natural intermediates,[10a,13] and speculations regarding involvement of sterol peroxides or hydroperoxides have been made.[14] In view of experimental evidence for the participation of cholesterol 20α-hydroperoxide in the biosynthesis of 3β-hydroxpregn-5-en-20-one from cholesterol in the adrenal cortex,[15] the possibility that the Δ^7-6α-hydroperoxide **4b** serves as an intermediate in the bioconversion of **4a** to **12** should not be overlooked.

The radiation-induced oxidations of **2a, 3a,** and **4a** thus took different courses from one another, and only the oxidation of the Δ^7-3-alcohol **4a** followed the same direct course of abstraction of the allylic methylene hydrogens and formation of both epimeric allylic hydroperoxides without double bond migration, as found for cholesterol.[3] The marked preference for formation of the quasiequatorial 7β-hydroperoxide **1c** from cholesterol was not seen in the case of the isomeric sterols **2a, 3a,** and **4a.** Rather, more nearly equal amounts of epimeric hydroperoxides were formed. For **2a** the product ratio (**2b** + **2d**):**5b** was 1:1; for **3a** the ratio **1b:1c** was approximately 1:2; for **4a** the ratio (**4b** + **4d**):(**4c** + **4e**) was 3:5.

Radiation-induced oxidations of **2a, 3a,** and **4a** take different courses from their previously reported oxidations by singlet molecular oxygen. Reaction of the Δ^4-sterol **2a** with singlet molecular oxygen is complex,[16] with the Δ^4-3-ketone **5a** and 4α,5-epoxy-5α-cholestan-3-one (**13**) as major products.[16a] However, the variable amounts of **5a** found relative to the epoxy ketone **13**[16c-e] and our present demonstration of the ease with which **5a** is formed from **2a** by radiation-induced free-radical attack of molecular oxygen suggest that **5a** also arises from **2a** in photosensitized oxidations by hydrogen atom abstraction and free-radical attack of molecular oxygen and not by attack of singlet molecular oxygen.[17] Formation of the epoxy ketone **13** from singlet molecular oxygen attack on **2a** *via* the putative intermediate 3-hydroxy-5α-cholest-3-ene 5-hydroperoxide[16a,c] would then be the likely event.

Attack of singlet molecular oxygen on the Δ^6-sterol **3a** and on the 3β-acetate of **3a** yielded the expected Δ^5-7α-hydroperoxide **1b** and the 3β-acetate of **1b,** respectively, as major product.[19] Small amounts of epimeric 7β-hydroperoxide **1c** were also apparently formed but not noticed.[19] By contrast free-radical oxidation of **3a** gave the same epimeric Δ^5-7-hydroperoxides **1b** and **1c** but with the quasiequatorial 7β-hydroperoxide **1c** predominating.

Singlet molecular oxygen attack on the 3β-acetate of the Δ^7-sterol **4a** proceeded by abstraction of the 14α-hydrogen and formation of 3β-acetoxy-5α-cholest-8(14)-ene 7α-hydroperoxide (**14**) as initial product, which itself was oxidized by singlet molecular oxygen to yield 3β-acetoxy-5α-cholest-14-ene 7α,8α- and 7α,8β-dihydroperoxides.[16c] The Δ^7-sterol **4a** also consumed two molecules of oxygen in photosensitized oxidations, but products were not isolated. It would seem likely that the same course of oxidation be taken for both **4a** and its 3β-acetate however. The point of attack and the sensitivity to further oxidation of the initially formed hydroperoxide are thus in total distinction to the behavior of **4a** in radiation-induced free-radical oxidations.

These results establish that the Δ^7-sterol **4a** as well as cholesterol, but not the Δ^4- and Δ^6-sterols **2a** and **3a,** may serve as probes in test of the electronic excitation state of molecular oxygen involved in chemical and enzymic reactions.

Experimental Section[20]

Irradiation Conditions. Pure crystalline samples of the parent sterols **2a, 3a,** and **4a** were irradiated in glass beakers open to the air for 16–20 hr (2.7×10^5 rads/hr) with ^{60}Co γ radiation from a Gammacell 200 (Atomic Energy of Canada Ltd., Ottawa), after which time the samples were recrystallized from methanol–diethyl ether. Recovered parent sterol free from detectable oxidation products was again irradiated and recrystallized. Irradiation and recrystallization were repeated several times to provide adequate amounts of oxidation products. Mother liquors containing oxidation products were evaporated under vacuum, and the combined residues were chromatographed on 1.0-mm thick chromatoplates using benzene–ethyl acetate (1:1) with triple development. Sterol components were detected and characterized by their thin-layer chromatographic mobility factors R (*vs.* the indicated parent sterol **2a, 3a,** or **4a** as unit mobility), color response to sulfuric acid spray, sterol peroxide test, and ultraviolet light absorption. Each resolved component was excised from the chromatoplate and eluted with acetone for further purification and for identification.

Pyrolysis Conditions. Analytical gas chromatography of **2b, 4b, 4c, 4d, 4e, 5b,** and **12** was conducted in the usual manner[7a,b,22] to obtain characteristic patterns of pyrolysis products. Gas chromatography of 1-mg samples, dissolved in 200 μl of acetone, was conducted for collection of all pyrolysis products in a single glass capillary.[21] The pyrolysis products were then chromatographed on a chromatoplate using benzene–ethyl acetate (1:1) with triple development and visualization in the same manner described for irradiation products. Pyrolysis products were eluted with acetone and recovered by evaporation of the solvent under vacuum for further purification and for identification.

Oxidation of Cholest-4-en-3β-ol (2a). From 75.3 mg of **2a,** mp 132–133°, irradiated and recrystallized, there was recovered 58.1 mg of pure **2a,** mp 131–133° (lit. mp 128–132°[23a]). Chromatography of the mother liquors resolved five components characterized and identified as follows: (a) R 1.13, yellow color, ultraviolet light absorbing, **5a;** (b) R 1.06, yellow color, positive peroxide test, ultraviolet light absorbing, **5b;** (c) R 1.00, pink color, **2a;** (d) R 0.72, tan color, positive peroxide test, **2b;** (e) R 0.56, tan color, positive peroxide test; and (f) R 0.32, tan color **2d.**

Cholest-4-en-3-one (5a). Fraction a derived from irradiation of **2a** was recovered and crystallized from methanol, yielding 2.1 mg of **5a,** mp 79–81° (lit. mp 76–82°[23a]); t_R 2.33; identical with authentic **5a** by uv, ir, tlc, and gc comparisons.

Irradiation of pure **5a** for 24 hr with ^{60}Co radiation did not afford chromatographically detectable alteration products.

6β-Hydroperoxycholest-4-en-3-one (5b). Fraction b derived from **2a** recovered from the acetone eluate yielded 1.0 mg of **5b,** homogeneous by thin-layer chromatography, identified by its characteristic pyrolysis pattern with components at t_R 0.38, 1.48, 1.98 (**2e**), 6.65 (**8**), and 7.14 (**7**). Identity of **5b** was further established by reductions to **2e** and to **5c.**

Irradiation of 0.2 mg of **2c** for 18 hr yielded a mixture of products, chief among which were **2e, 6,** and **5b,** all identified by their thin-layer chromatographic properties.

Cholest-4-ene-3β,6β-diol (2e). A small amount of **5b** (derived from **2a**) in methanol was reduced with an excess of sodium borohydride, yielding the 3β,6β-diol **2e,** R 0.31 in benzene–ethyl acetate (1:1); steel blue color with 50% sulfuric acid; pyrolysis pattern t_R 0.44, 0.81. and 2.00; identical in these properties with those of an authentic sample of **2e.**[5]

6β-Hydroxycholest-4-en-3-one (5c). A small sample of **5b** (derived from **2a**) was reduced with sodium iodide and acetic acid, yielding **5c,** R 0.62 in benzene–ethyl acetate (1:1); yellow color with 50% sulfuric acid; pyrolysis pattern t_R 6.65 (**8**) and 7.14 (**7**); identical in these properties with those of an authentic reference sample of **5c.**

3β-Hydroxycholest-4-ene 6α-Hydroperoxide (2b). Fraction d derived from **2a** yielded 0.7 mg of **2b,** homogeneous by thin-layer chromatography, identified as such by its characteristic pyrolysis pattern on gas chromatography: t_R 1.48, 1.98 (**2d**), 6.66 (**8**), and 7.14 (**7**), and by reduction to **2d.**

Cholest-4-ene-3β,6α-diol (2d). (A) From 2a. Fraction f from irradiated **2a** yielded 0.3 mg of **2d,** identified as such by its chromatographic properties R 0.32 in benzene–ethyl acetate (1:1); tan color with 50% sulfuric acid; pyrolysis pattern t_R 0.44, 0.80, and 2.04; identical in these properties with those of an authentic reference sample of **2d.**[5]

(B) From 2b. A small sample of **2b** derived from irradiation of **2a** was reduced with an excess of sodium borohydride in methanol, yielding the 3β,6α-diol **2d,** R 0.32 in benzene–ethyl acetate (1:1); tan color with 50% sulfuric acid; pyrolysis pattern t_R 0.44, 0.81, and 2.04; identical in these properties with those of an authentic reference sample of **2d.**[5]

Fraction e derived from **2a** was recovered on evaporation of the acetone eluates, yielding 0.2 mg of an unidentified sterol peroxide characterized by a characteristic pyrolysis pattern t_R 0.37, 1.92, and 2.66. From fraction c 5.3 mg of unaltered parent sterol **2a,** mp 130–133°, was recovered.

Oxidation of 5α-Cholest-6-en-3β-ol (3a). Irradiation of a 3.2-mg pure crystalline sample of **3a** (free from **1a** and other detectable sterols) was conducted for 20 hr. Direct thin-layer chromatography of the entire irradiated sample without prior recrystallization to recover **3a** resolved six zones: (a) R 1.00, orange-red color, **3a;** (b) R 0.74, instant blue color, positive peroxide test, **1b** and **1c;**

(c) R 0.62, no color, ultraviolet light absorbing, **9**; (d) R 0.53, instant blue color, **3c** (trace only); (e) R 0.42, instant blue color, **1e**; (f) R 0.35, instant blue color, **1d**.

3β-Hydroxycholest-5-ene 7-Hydroperoxides (1b and 1c). Fraction b derived from **3a** recognized to contain **1b** and **1c** by thin-layer chromatography was reduced with sodium borohydride in methanol, and the product 3β,7-diols **1d** and **1e** were identified as such by additional thin-layer and gas chromatographic analyses. Based on relative intensity of color response of the 3β,7-diols **1d** and **1e** to 50% sulfuric acid spray the 7β-hydroperoxide **1e** was present in approximately twice the amount as **1d**. No **3c** indicative of the presence of **3b** among the hydroperoxide products from **3a** was detected despite a careful search.

Identity of fractions c, d, e, and f from **3a** containing **9, 3c, 1e,** and **1d,** respectively, was achieved by additional thin-layer and gas chromatographic analyses. Behavior identical with that of authentic reference sterols was obtained in each case. Identity of recovered **3a** was carefully checked by thin-layer chromatography using benzene–ethyl acetate (7:3) with triple ascending development, which technique resolved **3a** (R 0.93) from **1a** (R 1.00). No **1a** was detected in **3a** recovered from fraction a.

A 2.1-mg sample of **3b** was irradiated for 20 hr. Analysis of the sample indicated that approximately 90% of the 5α-hydroperoxide **3b** was unaltered and that only approximately a 10% conversion of **3b** to a mixture of **1b** and **1c** had occurred. Traces of the epimeric 3β,7-diols **1d** and **1e** and of the 3β,5α-diol **3c** were also detected.

Oxidation of 5α-Cholest-7-en-3β-ol (4a). From 715 mg of pure **4a,** mp 120–122°, free from **12,** irradiated, a total of 215 mg of **4a,** mp 119–121° (lit. mp 118–127°[23a]), was recovered by recrystallization. The combined mother liquors were complex, but nine discrete zones were resolved for characterization and recovery work, as follows: (a) R 1.25, purple color, containing several components, not examined further; (b) R 1.00, tan turning purple color, **4a**;[24] (c) R 0.85, tan color, positive peroxide test, **4c**;[24] (d) R 0.81, tan color, positive peroxide test, **4b**;[24] (e) R 0.77, yellow color, ultraviolet light absorbing, **11**; (f) R 0.60, tan color, positive peroxide test, not examined further; (g) R 0.50, tan color, positive peroxide test, not examined further; (h) R 0.35, tan color turning purple, **4e**; (i) R 0.30, tan color turning purple, **4d**.

3β-Hydroxy-5α-cholest-7-ene 6α-Hydroperoxide (4b). Fraction d derived from **4a** was rechromatographed several times using benzene–ethyl acetate (1:1) with triple development. Elution of the R 0.81 component with acetone yielded 6.8 mg of **4b,** homogeneous by thin-layer chromatography but which could not be crystallized. The 6α-hydroperoxide **4b** was characterized by thin-layer chromatographic mobility, R 0.81, in benzene–ethyl acetate (1:1) and by a characteristic pyrolysis pattern which included major components at t_R 1.21, 3.08, 4.07, 5.68, 6.17, and 7.18.

3β-Hydroxy-5α-cholest-7-ene 6β-Hydroperoxide (4c). From fraction c crude **4c** was recovered which was rechromatographed several times on both 0.25 and 1.0 mm thick chromatoplates using the system benzene–ethyl acetate (1:1) with triple development, yielding 13.4 mg of pure **4c.** Recrystallization of the sterol from methanol gave the analytical sample of **4c,** mp 145–149°: $\tilde{\nu}_{max}$ (KBr) 3375, 3150, 1650, and 1055 cm^{-1}; pyrolysis pattern t_R 1.20, 3.00, 4.06, 5.69, 6.16, 7.17.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.74; H, 10.89.

A commercially obtained sample of **4a** stored as received for some months in the laboratory was recrystallized from methanol–diethyl ether, and the mother liquors were chromatographed on thin-layer chromatoplates using benzene–ethyl acetate (1:3) with double development. The parent sterol **4a** was recovered from a zone at R_f 0.71. The major peroxide zone at R_f 0.64 was identified as **4c** by additional thin-layer and gas chromatography. A third component at R_f 0.57 was identified as the Δ^7-6-ketone **11** by thin-layer and gas chromatography.

5α-Cholest-7-ene-3β,6α-diol (4d). (A) From 4a. Fraction i from irradiation of **4a** was eluted and the sterol recrystallized from methanol, yielding 8.5 mg of pure **4d,** mp 186–188° (lit. mp 192°;[25a] mp 178–179° and 185–186°;[13b] mp 114°[25b]); pyrolysis pattern t_R 1.20, 1.49, 2.88, and 3.08; identical with authentic **4d** prepared by hydroboration of **12**[25a] by ir, tlc, and gc comparisons.

(B) From 4b. A solution of 3.5 mg of **4b** in methanol was treated with an excess of sodium borohydride and the product recovered, yielding 2.7 mg of **4d** as colorless crystals, mp 184–188°; identical in spectral and chromatographic properties with an authentic reference sample of **4d.**

(C) From Pyrolysis. Pyrolysis of **4b, 4c, 4d,** and **4e** yielded a component recognized as **4d** by thin-layer chromatography. Elution and thin-layer chromatography confirmed the presence of **4d,** further recognized by its characteristic pyrolysis pattern which included components at t_R 1.20 (**12**), 1.49, 2.88, and 3.08 (**4d**).

5α-Cholest-7-ene-3β,6β-diol (4e). (A) From 4a. Fraction h from irradiation of **4a** was eluted and recrystallized from methanol, yielding 11.3 mg of **4e,** mp 204–207° (lit. mp 207–209°;[13a] mp 204–207°[13b]); pyrolysis pattern t_R 1.19, 1.48, and 3.00; identical with authentic **4e** prepared by sodium borohydride reduction of **11**[13b] by ir, tlc, and gc comparisons.

(B) From 4c. A solution of 3.4 mg of **4c** in methanol was reduced with an excess of sodium borohydride and the product recovered, yielding 2.3 mg of pure **4e,** mp 205–207°; identical in spectral and chromatographic properties with those of an authentic reference sample of **4e.**

(C) From Pyrolysis. Pyrolysis of **4c** and **4e** yielded a component recognized as **4e.** Elution from the chromatoplate and additional thin-layer chromatography confirmed identity as **4e,** further recognized by its characteristic pyrolysis pattern which included components at t_R 1.19 (**12**), 1.48, and 3.00 (**4e**).

3β-Hydroxy-5α-cholest-7-en-6-one (11). (A) From Irradiation of 4a. Fraction e obtained by irradiation of **4a** was rechromatographed on 1.0 mm thick chromatoplates using chloroform–acetone (23:2) with triple ascending development. The ultraviolet light absorbing zone was eluted with acetone, yielding 28.5 mg of **11,** mp 195–197° (lit. mp 196–197°;[26] mp 162–164°[13b]); t_R 4.07, 5.69, 6.17, and 7.16; identical with authentic **11** by uv, ir, tlc, and gc comparisons.

(B) From Pyrolysis of 4b, 4c, and 4e. Pyrolysis of **4b, 4c,** and **4e** gave in each case a component with thin-layer chromatographic properties of **11.** Elution of the component gave chromatographically homogeneous **11,** identified by comparison of spectral and chromatographic properties with those of an authentic reference sample of **11.**

Cholesta-5,7-dien-3β-ol (12). (A) From Pyrolysis of 4b, 4c, 4d, and 4e. The major pyrolysis product from **4b, 4c, 4d,** and **4e** was eluted from the thin-layer chromatoplate with acetone and crystallized from methanol, yielding **12,** typically characterized by mp 148–150° (lit. mp 142–150°[23a]); t_R 1.20; identical with authentic **12** by uv, ir, tlc, and gc comparisons.

(B) From Irradiation of 4a. Fraction b obtained by irradiation of **4a** was eluted with acetone and crystallized from methanol, yielding 228 mg of **4a,** mp 119–122° (lit. mp 118–127°[23a]), recognized as containing the 5,7-diene **12** as contaminant by chromatographic and spectral data. Thin-layer chromatography of the recovered **4a** using benzene–ethyl acetate (6:1) with triple development resolved **4a** at R_f 0.66 (tan color turning purple with 50% sulfuric acid) and **12** at R_f 0.70 (steel blue color). Absorption of the recovered **4a** fraction exhibited λ_{max}(EtOH) 271.5, 282, and 293 nm characteristic of **12.** From the absorbance of the 282-nm band approximately 0.3% of **12** in the recovered **4a** sample was indicated.

Acknowledgment. The authors gratefully acknowledge the aid of Professor G. O. Schenck, Max Planck Institute, Mülheim a.d. Ruhr, in providing a copy of the dissertation of Dr. W. Eisfeld.

Registry No.—**1b,** 2846-29-9; **1c,** 36871-91-7; **2a,** 517-10-2; **2b,** 41-209-89-6; **2d,** 15013-60-2; **2e,** 570-88-7; **3a,** 22420-06-0; **4a,** 80-99-9; **4b,** 52718-87-3; **4c,** 52555-46-1; **4d,** 2259-91-8; **4e,** 2259-90-7; **5a,** 601-57-0; **5b,** 2207-76-3; **5c,** 570-89-8; **11,** 14858-06-1; **12,** 434-16-2.

References and Notes

(1) Paper XXXI of the series: Y. Y. Lin and L. L. Smith, *J. Label. Compounds,* in press. Financial support of these studies was kindly provided by the Robert A. Welch Foundation, Houston, Tex.
(2) Robert A. Welch Foundation Postdoctoral Fellow, 1972–1974.
(3) L. L. Smith, J. I. Teng, M. J. Kulig, and F. L. Hill, *J. Org. Chem.,* **38,** 1763 (1973).
(4) J. I. Teng, M. J. Kulig, L. L. Smith, G. Kan, and J. E. van Lier, *J. Org. Chem.,* **38,** 119 (1973).
(5) M. J. Kulig and L. L. Smith, *J. Org. Chem.,* **38,** 3639 (1973).
(6) (a) J. I. Teng and L. L. Smith, *J. Amer. Chem. Soc.,* **95,** 4060 (1973); (b) L. L. Smith and J. I. Teng, *ibid.,* **96,** 2640 (1974).
(7) (a) J. I. Teng, M. J. Kulig, and L. L. Smith, *J. Chromatogr.,* **75,** 108 (1973); (b) L. L. Smith, M. J. Kulig, and J. I. Teng, *Steroids,* **22,** 627 (1973).
(8) L. L. Smith and F. L. Hill, *J. Chromatogr.,* **66,** 101 (1972).
(9) (a) G. J. Schroepfer and I. D. Frantz, *J. Biol. Chem.,* **236,** 3137 (1961);

(b) M. E. Dempsey, J. D. Seaton, G. J. Schroepfer, and R. W. Trockman, *ibid.*, **239**, 1381 (1964); (c) M. E. Dempsey, ibid., **240**, 4176 (1965).
(10) (a) S. M. Dewhurst and M. Akhtar, *Biochem. J.*, **105**, 1187 (1967); (b) M. Akhtar and M. A. Parvez, *ibid.*, **108**, 527 (1968).
(11) (a) A. M. Paliokas and G. J. Schroepfer, *Biochem. Biophys. Res. Commun.*, **26**, 736 (1967); (b) A. M. Paliokas and G. J. Schroepfer, *J. Biol. Chem.*, **243**, 453 (1968); (c) M. Akhtar and S. Marsh, *Biochem. J.*, **102**, 462 (1967).
(12) D. J. Aberhart and E. Caspi, *J. Biol. Chem.*, **246**, 1387 (1971).
(13) (a) W. E. Harvey and K. Bloch, *Chem. Ind. (London)*, 595 (1961); (b) M. Slaytor and K. Bloch, *J. Biol. Chem.*, **240**, 4598 (1965).
(14) (a) A. M. Paliokas and G. J. Schroepfer, *Biochim. Biophys. Acta*, **144**, 167 (1967); (b) A. Fiecci, M. Galli Kienle, A. Scala, G. Galli, R. Paoletti, and E. Grossi Paoletti, *J. Biol. Chem.*, **247**, 5898 (1972).
(15) (a) J. E. van Lier and L. L. Smith, *Biochim. Biophys. Acta*, **210**, 153 (1970); **218**, 320 (1970); (b) J. E. van Lier and L. L. Smith, *Biochem. Biophys. Res. Commun.*, **40**, 516 (1970).
(16) (a) A. Nickon and W. L. Mendelson, *J. Org. Chem.*, **85**, 1894 (1963); **87**, 3921 (1965); (b) E. J. Corey and W. C. Taylor, *J. Amer. Chem. Soc.*, **86**, 3881 (1964); (c) W. Eisfeld, Dissertation, Göttingen, 1965, p 63; (d) D. R. Kearns, R. A. Hollins, A. U. Khan, R. W. Chambers, and P. Radlick, *J. Amer. Chem. Soc.*, **89**, 5455 (1967); (e) D. R. Kearns, R. A. Hollins, A. U. Khan, and P. Radlick, *ibid.*, **89**, 5456 (1967); (f) R. W. Murray and M. L. Kaplan, *ibid.*, **91**, 5358 (1969).
(17) Formation of **5a** from **2a** suggested as arising from participation of the $^1\Sigma_g^+$ excited state of molecular oxygen rather than the first $^1\Delta_g$ excited state[16d,e] has since been qualified.[18] Although hydrogen atom abstraction by excited dye molecules had previously not been thought likely,[16e] free-radical oxidation initiated by the $^3(n,\pi^*)$ sensitizer has recently been posited.[18a]
(18) (a) K. Gollnick, T. Franken, G. Schade, and G. Dörhöfer, *Ann. N. Y. Acad. Sci.*, **171**, 89 (1970); (b) C. S. Foote, *ibid.*, **171**, 105 (1970); (c) D. R. Kearns, *ibid.*, **171**, 106 (1970).
(19) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **81**, 6330 (1959); **83**, 1498 (1961).
(20) Melting points were taken on a calibrated Kofler block under microscopic magnification. Infrared absorption spectra were recorded over the range 400–4000 cm^{-1} on 1.5 mm diameter KBr disks incorporating the sample using a Perkin-Elmer Model 337 spectrophotometer equipped with a beam condenser lens. Ultraviolet light absorption spectra were recorded on 95% ethanol solutions using a Cary Model 14 spectrophotometer. Thin-layer chromatography was conducted with 20 × 20 cm chromatoplates of silica gel HF_{254} (E. Merck GmbH., Darmstadt), 0.25 and 1.0 mm thick, using specified solvent systems. Thin-layer mobility factors *R* for products derived from parent sterols **2a**, **3a**, or **4a** were measured using the appropriate parent sterol as unit mobility. Sterols were detected by viewing under 254-nm light, followed by spraying with *N,N*-dimethyl-*p*-phenylenediamine for peroxides,[8] and finally by spraying with 50% aqueous sulfuric acid and heating for full color display. Gas chromatography was conducted on 1.83 m long × 4 mm diameter silanized glass U-tubes packed with 3% SP-2401 on 100–120 mesh Supelcoport (Supelco Inc., Bellafonte, Pa.) using a Hlewlett-Packard F&M Model 402 gas chromatograph equipped with a hydrogen flame ionization detector. Injection temperature was 250°; column temperature was 230°; detector temperature was 250°. Nitrogen was used as carrier gas at a flow rate of 20 ml/min. Retention time data (t_R) are expressed in terms of cholesterol as unit retention time in all cases. Preparative gas chromatography was achieved by collection of effluxing components in glass capillaries as previously described.[21]
(21) J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **36**, 7 (1968).
(22) J. E. van Lier and L. L. Smith, *Steroids*, **15**, 485 (1970).
(23) Literature melting point and ultraviolet light absorption data for the common steroids were obtained, respectively, from (a) J. Jacques, H. Kagan, and G. Ourisson, "Tables of Constants and Numerical Data," Vol. 14, Pergamon Press, Oxford, 1965; (b) L. Dorfman, *Chem. Rev.*, **53**, 47 (1953).
(24) Chromatographic resolution and differentiation by color display of the parent sterols **1a** and **4a** as well as of their major hydroperoxides formed by radiation-induced oxidation are achieved using benzene–ethyl acetate (18:7), as follows: **1a**, red color, *R* 1.00; **4a**, orange-red color, *R* 0.91; **1b**, instant blue color, *R* 0.52; **1c**, instant blue color, *R* 0.57; **4b**, tan color, *R* ~0.37; **4c**, tan color, *R* 0.39. The chief product **3a** of singlet molecular oxygen attack on **1a** is not resolved from **1b** but the corresponding 3β,5α-diol **3c** is readily resolved from **1d** and from **1e** in a variety of solvent systems. The putative initial product of singlet molecular oxygen attack on **4a** as well as the secondary dihydroperoxides formed is resolved from **4b** and **4c** using multiple irrigation with benzene–ethyl acetate (18:7).
(25) (a) L. Caglioti, G. Cainelli, and G. Maina, *Tetrahedron*, **19**, 1057 (1963); (b) D. J. Aberhart, J. G. Lloyd-Jones, and E. Caspi, *Phytochemistry*, **12**, 1065 (1973).
(26) L. Tökés, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 5465 (1968).

A Reexamination of the Origin of Regioselectivity in the Dimerization of Acrolein. A Frontier Orbital Approach

Peter V. Alston*

E. I. du Pont de Nemours & Company, Analytical Research Group, Spruance Plant, Richmond, Virginia 23261

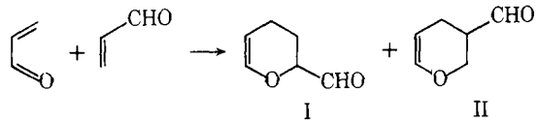
Donald D. Shillady

Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284

Received March 18, 1974

The relative frontier orbital coefficient magnitudes of acrolein were determined from *ab initio* SCF molecular orbitals. These frontier orbital coefficients favor the experimentally observed regioisomer in the dimerization of acrolein. Various all valence electron semiempirical SCF MO methods agree with the *ab initio* calculations on the origin of regioselectivity in the reaction. First-order charge interactions were not useful in predicting the regioselectivity of the reaction. Generalized rules for the prediction of the regioselectivity in cycloadditions involving three terminal carbon atoms and one terminal oxygen atom are given.

The regioselectivity in the dimerization of acrolein has been of theoretical interest [1-5] over the last several years. Salem[3] found that Hückel orbital interactions favored regioisomer II whereas only regioisomer I occurs experimentally. A later calculation by Devaquet and Salem[4] using π



I II

SCF MO's and including first-order charge interactions as well as overlap was found to be in agreement with the experimental results. However, the major contribution (60–70%) to the stabilization of I relative to II arose from the electrostatic term. This is confusing because consideration of the π charge densities of acrolein would lead to the wrong prediction.[1] There is also some question about the reliability of the overlap energy term in this calculation because it predicts that the exo approach is more stable than the endo for regioisomer II. This prediction is contrary to orbital symmetry considerations[6,7] and experimental evidence,[8] which indicate that the endo configuration is more stsble than the exo. Later, Eisenstein, *et al.*,[1] was successful in predicting the observed regioisomer I using a frontier orbital approach based on Hückel orbitals. However, Houk[2] has recently found that various molecular orbital methods disagree on the relative coefficient magnitudes of the HOMO of acrolein. Consequently, the origin of the regioselectivity in the dimerization of acrolein is presently unclear. In this paper, we have reexamined this reaction