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Ozonization of Cholesterol[1]

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Abstract: The ozonization of cholesterol in aqueous dispersion gave minor products 5,6α-epoxy-5α-cholestan-3β-ol and 5,6β-epoxy-5β-cholestan-3β-ol and major product 5ξ,6ξ-epidioxy-5,6-secocholestane-3β,5ξ,6ξ-triol, along with 3β-hydroxy-5-oxo-5,6-secocholestan-6-al and 3β,10-dihydroxy-5,6:5,10-disecocholestan-5-oic acid lactone (5→10) derived by decomposition of the major epidioxide product. Acetic anhydride/pyridine treatment of the epidioxide resulted in rearrangement, yielding 3β-acetoxy-10-hydroxy-6-oxo-5,6;5,10-disecocholestan-5-oic acid lactone (5→10), 10-hydroxy-6-oxo-5,6:5,10-disecocholest-3-en-5-oic acid lactone (5→10), and 6,6-diacetoxy-10-hydroxy-5,6;5,10-disecocholest-3-en-5-oic acid lactone (5→10). Acetylation of homologue 5ξ,6ξ-epidioxy-6ξ-methoxy-5,6-secocholestane-3β,5ξ-diol and its 3β-acetate also involved rearrangement to disecolactones, including 3β,6ξ-diacetoxy-10-hydroxy-6ξ-methoxy-5,6:5,10-disecocholestan-5-oic acid lactone (5→10). These results establish that 5,6-secosterol formation is the major ozonization process and 5,6-epoxidation a minor independent process and that "anomalous" lactone products derive by rearrangement of initially formed epidioxides.

The action of ozone (O_3) on cholesterol (**1a**) (Chart I) in organic solvents has received attention repeatedly from 1905, and there is general agreement that 5,6-secosterols result.[3,4] However, a satisfactory description of the process and of products has not been recorded. A poorly defined ozonide $C_{27}H_{46}O_4$ (**2a**) is suggested, the reduction (Zn/acetic acid or Raney Ni) of which yields 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (**3**).[4b,d] Similarly, ozonization of cholesterol 3β-acetate (**1b**) gives a poorly characterized ozonide 3β-acetate **2b** reducible to the corresponding secoaldehyde **3** 3β-acetate.[4f,5] More vigorous ($LiAlH_4$) reductions of ozonides **2**, secoaldehyde **3** 3β-acetate, or 3β-hydroxy-5-oxo-5,6-secocholestan-6-oic acid (**4**) 3β-acetate gave 5β-5,6-secocholestane-3β,5α,6-triol (**5**).[4e,6] Oxidation of ozonide **2a** and piperidine-induced rearrangement of **2b** gave secoacids **4** and **4** 3β-acetate, respectively.[4d,7]

Solvent participation occurs in ozonizations of cholesterol or **1b** conducted in protic solvents. In halocarbons containing methanol, 5ξ,6ξ-epidioxy-6ξ-methoxy-5,6-secocholestane-3β,5ξ-diol (**6b**) or **6b** 3β-acetate result.[4e,8] Reduction of **6b** 3β-acetate with Zn/acetic acid gave **3** 3β-acetate,[8] and with $LiAlH_4$ gave the secotriol **5**.[4e] Furthermore, cholesterol appears to be ozonized in aqueous media,[9] and cholesterol ozonide preparations formed in aprotic media appear to react in water to yield H_2O_2, CO_2, aldehyde, and acid.[3c,d,9a]

In completion of studies of the oxidation chemistry of cholesterol with defined oxygen species,[10] we have reexamined the action of O_3 on cholesterol. Our aim is to identify all products and processes implicated by using effective chromatographic methods, a means not fully exploited heretofore in studies of ozonization of olefins.[11]

Results

The ozonization of cholesterol (or **1b**) in nonparticipating organic solvents CCl_4, chloroform (freed from stabilizing ethanol), or methylene chloride at dry ice temperature yielded a complex mixture of products more mobile than cholesterol (or **1b**) on chromatograms. Only in ethyl acetate were more polar products found, these being the isomeric 5,6-epoxides 5,6α-epoxy-5α-cholestan-3β-ol (**7a**) and 5,6β-epoxy-5β-cholestan-3β-ol (**7b**).[12]

The more polar secoaldehyde **3** was not detected by chromatography among ozonization products, but strong 1720-cm^{-1} absorption characterized isolated crude products.[13] However, reduction of the crude products with Zn/acetic acid gave secoaldehyde **3** as major product, as expected.[4b,d] Reduction with $LiAlH_4$ gave secotriol **5**. These results confirm that cholesterol reacts with O_3 in nonparticipating solvents to form 5,6-secosterol products, but other than demonstrating that such reactions yield initial products different from those obtained in participating protic solvents, we have not pursued these analyses.

Reaction of cholesterol or **1b** with O_3 in chloroform containing methanol or ethanol at dry ice temperature involved solvent participation. The more mobile products formed in neat chloroform were not observed, and formation of the alkoxyperoxides **6b** or **6c** (**6b** or **6c** 3β-acetates from **1b**) resulted in exact confirmation of the prior work of Lettrē.[4e] In neat *tert*-butyl alcohol at room temperature, 6ξ-*tert*-butoxy-5ξ,6ξ-epidioxy-5,6-secocholestane-3β,5ξ-diol (**6d**) was formed from cholesterol. The isomeric 5,6-epoxides **7** were minor products.

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[12] Ozonization of **1b** in methylene chloride is also reported to yield **7b** 3β-acetate.[4f]

[13] Similar 1712–1720-cm^{-1} absorption (in addition to 1740-cm^{-1} absorption of the acetate carbonyl) was previously recorded by Lettrē and Jahn[4e] as characterizing isolated crude ozonization products of **1b**.

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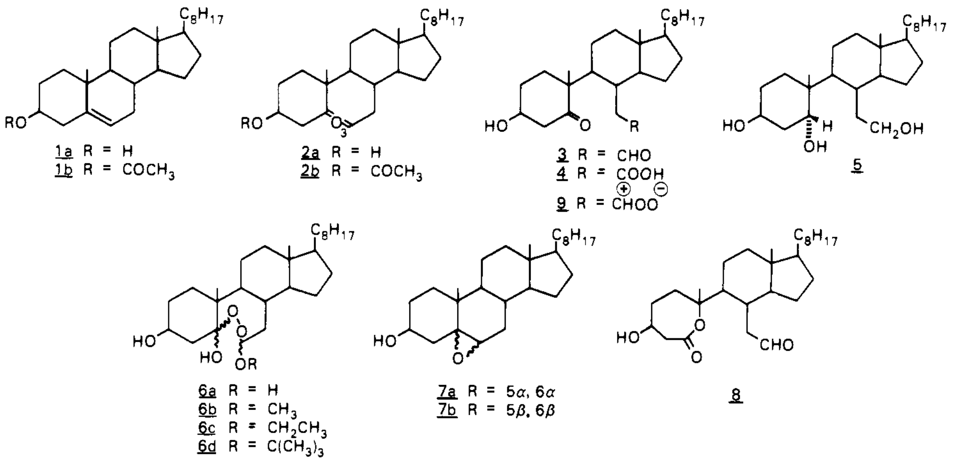
Chart I



The preference for reaction with alcohol demonstrated by Lettre in ozonizations of **1b** extended to the formation of **6c** 3β-acetate in ozonizations conducted in chloroform containing stabilizing ethanol,[4e] and we demonstrate here the same avidity of ozonized cholesterol for reaction with ethanol. Furthermore, the preference for reaction with alcohol is observed in aqueous systems discussed shortly hereafter.

Cholesterol in water dispersion was rapidly and completely oxidized at room temperature by O_3 to major peroxidic product 5ξ,6ξ-epidioxy-5,6-secocholestane-3β,5ξ,6ξ-triol (**6a**) and to minor products secoaldehyde **3**, isomeric 5,6-epoxides **7**, and lactone 3β,10-dihydroxy-6-oxo-5,6;5,10-disecocholestan-5-oic acid lactone (5→10) (**8**). Ozonization in 50% aqueous tetrahydrofuran solution gave the same yields of **3**, **6a**, and **7**. However, in 50% aqueous methanol the methoxyperoxide **6b** and 5,6-epoxides **7** were formed, with no peroxide **6a** nor secoaldehyde **3**. In 50% aqueous acetic acid, peroxide **6a** was formed, with no 5,6-epoxides **7**. No trace of a putative 6ξ-acetoxy-5ξ,6ξ-epidioxy-5,6-secocholestane-3β,5ξ-diol representing solvent acetic acid participation was detected.

The isomeric 5,6-epoxides **7** were repeatedly recovered as crystalline materials in a constant **7a**:**7b** ratio of 1:8; the same as that found in oxidations of cholesterol in water by air, sterol hydroperoxides, or H_2O_2.[10d,e,h] The 5,6-epoxides **7** are genuine products of reaction between cholesterol and O_3 as they are formed early in the reaction, at least as early as major product **6a**, and are not formed in control dispersions treated with H_2O_2 or sparged with O_2 (no O_3 generation). Moreover, hydroxyl radical potentially derived from O_3 in water[14] does not account for 5,6-epoxides **7** as other cholesterol oxidation products also formed by hydroxyl radical[10j] were not encountered.

The major product **6a** also did not oxidize cholesterol in aqueous dispersion or in pyridine or acetonitrile solutions, thus eliminating this process as accounting for derivation of the 5,6-epoxides **7** from cholesterol. It is possible that epoxides **7** form by attack on cholesterol of a putatively formed Criegee zwitterion,[15] 3β-hydroxy-5-oxo-5,6-secocholestane-6-carbonyl oxide (**9**) in the present case.

Identity of secoaldehyde **3** obtained only as a colorless oil[16] was established by spectral data given in the Experimental Section and by $LiAlH_4$ reduction to crystalline secotriol **5**.[17] Secoaldehyde **3** is clearly not a direct ozonization product but is a decomposition product of initially formed **6a**. Recovery of pure **6a** was a troublesome matter, as preparations were found to be contaminated with **3** unless careful precautions were taken. Storage of pure **6a** at room temperature or in the cold resulted in traces of **3** in the samples, and chromatography of **6a** on silica gel resulted in the conversion of **6a** to **3**. Adsorption on silica gel for 24 h resulted in 70–80% conversions to **3**, for 3 h in 5–10% conversions. Only rapid high-performence liquid chromatography of **6a** preparations served to give **6a** free of **3**; storage in a freezer reduced the transformation of **6a** to **3**. Furthermore, the direct transformation of **6a** to **3** was observed. Although solutions of pure **6a** in anhydrous methanol or acetic acid were relatively stable, addition of a drop of water caused the conversion of **6a** to **3** within 48 h.

The slow autoxidation of secoaldehyde **3** to the corresponding secoacid **4** was observed for **3** left exposed to air.

Careful examination of chromotographic and proton spectral data evinced the presence of but one stereoisomeric peroxide **6** in each experiment,[18] even though both isomeric 5,6-epoxides **7** were formed. The 5,6-secosterol structure of the major product **6a** was established by its reduction by $NaBH_4$ to secoaldehyde **3** and by $LiAlH_4$ to secotriol **5**. The 5,6-peroxydiol feature is assigned from spectral data rendered in detail in the Experimental Section and by analogy to the alkoxylperoxy structures of **6b** and **6c** established definitively herein. Crucial evidence demonstrating the structure is a one-proton triplet signal at 5.22 ppm in the proton spectrum of **6a** (4.60–4.72 ppm for **6b**, **6c**, and their 3β-acetates) arising from the 6ξ-proton geminal to two oxygen functions.[19] Moreover, **6a** is not formed by treatment of ozonide preparations **2a** with water nor altered by vacuum drying or by recrystallization from methanol (**6b** not thereby derived). These items support the assigned structure and eliminate isomeric alternatives such as **2a** hydrate and 5ξ-hydroperoxy-3β,5ξ-dihydroxy-5,6-secocholestan-6-al structures.

The 6-alkoxy-5,6-epidioxy-5-hydroxy features of **6b**, **6c**, and their 3β-acetates previously assigned on general chemical principles[4e] are explicitly established by spectra recorded in detail in the Experimental Section, as is also the structure of the 6ξ-*tert*-butoxy analogue **6d**. The key 6ξ-proton signal establishes attachment of the alkoxyl group at C-6 and not at the alternative C-5 possibility. The 6-methoxy group of **6b** is also demonstrated by retention of the 6-methyl ether feature in 3β,6ξ-diacetoxy-

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[14] Alder, M. G.; Hill, G. R. *J. Am. Chem. Soc.* **1950**, *72*, 1884–1886.

[15] (a) Hinrichs, T. A.; Ramachandran, V.; Murray, R. W. *J. Am. Chem. Soc.* **1979**, *101*, 1282–1284. (b) Adam, W.; Rodriguez, A. *Ibid.* **1980**, *102*, 404–406.

[16] Secoaldehyde **3** has also been described as a crystalline diethyl ether solvate: mp 55–60 °C.[4d]

[17] Secotriol **5** gave a 3β,6-diacetate with but traces of a triacetate, in agreement with Lettrē and Jahn who obtained only **5** diesters.[4e] Furthermore, the 5-ketone group of **3** and **3** 3β-acetate is unreactive toward carbonyl reagents.[4d,e] These items suggest the 5α (axial)-hydroxyl group of **5** be subject to steric hindrance of the sort exhibited in the well-known steroid 11β(axial)-hydroxyl case.

[18] Hop-17(21)-en-3β-ol and adian-5-ene also gave only one stereoisomeric ozonide upon ozonization in organic solvents, cf.: (a) Itokawa, H.; Tachi, Y.; Kamano, Y.; Iitake, Y. *Chem. Pharm. Bull.***1978**, *26*, 331–333. (b) Ageta, H.; Shiojima, K.; Kamaya, R.; Masuda, K. *Tetrahedron Lett.* **1978**, 899–900. However, ozonization on silica gel of 5α-cholestan-3β-ol 3β-acetate yielded both possible stereoisomeric ozonides; cf. (c) Wife, R. L.; Kyle, D.; Mulheirn, L. J.; Volger, H. C. *J. Chem. Soc., Chem. Commun.* **1982**, 306–307.

[19] The proton signal of the analogous hydrogen atom geminal to peroxide and hydroxyl groups in simple peroxydiols RCH(OH)-O-O-CH(OH)R has been found in the range 5.07–5.13 ppm; cf.: Budinger, P. A.; Mooney, J. R.; Graselli, J. G.; Frey, P. S.; Guttman, A. T. *Anal. Chem.* **1981**, *53*, 884–889.

10-hydroxy-6ξ-methoxy-5,6:5,10-disecocholestan-5-oic acid lactone (5→10) (**12**) (Chart II) derived by acetylation of **6b** discussed hereafter.[20]

The structures of **6b–d** and **6b** and **6c** 3β-acetates are further supported by ^{13}C spectra that reveal two deshielded signals recognized as arising from ^{13}C atoms adjacent to two oxygen atoms. Singlet signals at 111.7–115.8 ppm and doublet signals at 94.7–102.6 ppm ascribed to the C-5 and C-6 atoms, respectively, establish the assigned structures definitively.

Similarties among proton spectra of peroxides **6** and their 3β-acetates suggest that all are members of a class with the same A/B-ring stereochemistry. An axial conformation of the 3α-proton and thereby A-ring chair conformation are inferred from the half-widths (22–25 Hz) of the 3α-proton signals. Moreover, the triplet character of the 6ξ-proton signal in spectra of **6a–c** and **6b** and **6c** 3β-acetates infers that dihedral angles 6ξ-H/C-6/C-7/7α-H and 6ξ-H/C-6/C-7/7β-H be the same. The comparable doublet of doublets signal in the **6d** spectrum suggests departure from equal dihedral angles due to steric bulk of the 6ξ-*tert*-butoxy group. Finally, a one-proton doublet of doublets signal at 2.64–2.68 ppm in the spectra of **6a–c** and **6b** and **6c** 3β-acetates is ascribed to hydrogen deshielded by neighboring oxygen (probably one in the 5ξ,6ξ-epidioxide feature).

Considerations of Dreiding molecular models of peroxides **6**, imposing the constraints of equal dihedral angles and of a unique deshielded hydrogen atom, show that minimum steric interactions are accommodated in a structure with 5β,6β-epidioxide and 5α-hydroxyl features, with the equatorial 4α-hydrogen being uniquely deshielded.[21] The 4α-proton doublet of doublets collapsed to a doublet with the same geminal coupling constant upon irradiation at the frequency of the coupled vicinal 3α-proton, thus establishing the correctness of the assignment. As the eight-membered B ring is conformationally mobile, it is possible to construct Dreiding model conformers with equal 6ξ-H/C-6/C-7/7-H dihedral angles with the 6ξ-hydroxyl (alkoxyl) group cis or trans to the 5α-hydroxyl, thus in 6*R* or 6*S* configurations. No C-6 stereochemistry is assigned accordingly.

Variable amounts of disecolactone **8**, the fifth ozonization product of cholesterol in water, were recovered. Moreover, **8** was observed to form from **6a** under the same conditions in which **6a** was transformed to **3**, thus on silica gel and in moist methanol or acetic acid solutions.[22] The unstable nature of **8** and its low recoveries precluded complete characterization, but spectral data sufficed to identify **8** as the 3β-alcohol analogue of 3β-acetoxy-10-hydroxy-6-oxo-5,6:5,10-disecocholestan-5-oic acid lactone (5→10) (**8** 3β-acetate) obtained as major product in attempted acetylations of peroxides **6**.

Treatment of **6a** with acetic anhydride/pyridine resulted in complete transformation to three unexpected rearrangement products **8** 3β-acetate, 10-hydroxy-6-oxo-5,6:5,10-disecocholest-3-en-5-oic acid lactone (5→10) (**10**), and 6,6-diacetoxy-10-hydroxy-5,6:5,10-disecocholest-3-en-5-oic acid lactone (5→10) (**11**). Similar treatment of **6b**, **6b** 3β-acetate, and **6c** 3β-acetate also gave the disecolactones **8** 3β-acetate and **10**. Additionally, **6b** and **6b** 3β-acetate yielded 3β,6ξ-diacetoxy-10-hydroxy-6ξ-methoxy-5,6:5,10-disecocholestan-5-oic acid lactone (5→10) (**12**). Simple acetylated derivatives of **6**, indeed any peroxidic products, were not observed. As peroxides **6** were stable in neat pyridine, the transformations must result from the action of acetic anhydride in the system.

Disecolactones **8**, **8** 3β-acetate, and **10–12** were recognized as a class of new oxidized secosterols from proton spectra, the unique feature of which being the C-19 proton chemical shift of 1.37–1.50 ppm, substantially deshielded from that of parent peroxides **6** and secoaldehyde **3**.[23] Access to structure eluciation of the class was gained via infrared spectra, devoid of hydroxylic absorption but revealing carbonyl absorption bands suggesting four separate features. The aldehyde features of **8**, **8** 3β-acetate, and **10** evinced by 1730- and 2730-cm^{-1} bands were confirmed by aldehyde proton signals and aldehyde carbonyl signal at 203.59 ppm in the ^{13}C spectrum of **8** 3β-acetate.

Nonaldehydic α,β-unsaturated carbonyl features in **10** and **11** were indicated by 1660- and 1700-cm^{-1} bands, confirmed by 216-nm absorption,[24] coupled vinyl proton signals, and sp^2 carbon resonances at 123.78 and 144.35 ppm (olefinic) and 165.99 ppm (carbonyl) in the ^{13}C spectrum of **11**.[25] Acetate ester features indicated by 1755–1775- and 1245–1255-cm^{-1} bands were confirmed by proton spectra revealing acetate methyl proton signals for **8** 3β-acetate, **11**, and **12** and 3α-proton signals for **8** 3β-acetate

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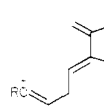
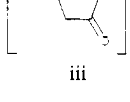
[20] The 6-alkoxy feature of **6b**, **6c**, and their 3β-acetates is also supported by prominent EI mass spectral ions *m/z* 263 for **6b** (principal ion for **6b** 3β-acetate) and *m/z* 227 for **6c** (principal ion for **6c** 3β-acetate) formulated as $(C_{18}H_{31}O)^+$ and $(C_{19}H_{33}O)^+$, respectively, thus homologues of one another. Speculative structures i and ii for these fragment ions are proposed.



i, *m/z* 263; R = CH_3
ii, *m/z* 277; R = C_2H_5

[21] Unique deshielded one-proton signals are reported in related steroids with 5α,6α-epidioxide and 5α,8α-epoxide features but devoid of C-5 hydroxylic substitution; cf.: Gumulka, J.; Szczepek, W. J.; Wielogorski, Z. *Tetrahedron Lett.* **1979**, 4847–4850, and ref 18b.

[22] Rearrangement of **6a** to **8** with concommitant scission of the C-9/C-10 bond also occurs in electron-impact mass spectrometry of **6a**. The principal ion *m/z* 143 in the **6a** spectrum is a doublet, one component arising from a $C_{11}H_{11}$ fragment (Calcd for $C_{11}H_{11}$: M, 143.086075. Found: M, 143.0864) and the other from the ion iii derived from the A ring of **8** (Calcd for $C_7H_{11}O_3$: M, 143.070805. Found: M, 143.0706).



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[23] C-19 proton signals of analogous steroid disecolactones have been found in the same region, thus 1.28–1.31 ppm for several ε-lactones of partial structures 10-hydroxy-5,6;5,10(or 4,5;5,10)-disecocholestan-5-oic acid lactone (5→10) and 10-hydroxy-3,5;5,10-diseco-*A*-norandrostan-5-oic acid lactone (5→10); and 1.32–1.36 ppm for γ-lactones 10-hydroxy-3,5;5,10-disecocholestane-3,5-dioic acid lactone (3→10) 5-methyl ester and 10,17-dihydroxy-3,5;5,10-diseco-*A*,*B*-bisnorandrostane-3,5-dioic acid lactone (3→10); cf.: (a) Caspi, E.; Balasubrahmanyam, S. N. *Experientia* **1963**, *19*, 396–397; *J. Org. Chem.* **1963**, *28*, 3383–3386. (b) Ahmad, M. S.; Shafiullah; Mushfiq, M. *Aust. J. Chem.* **1974**, *27*, 2693–2696. (c) Ahmad, M. S.; Waris, F. *Indian J. Chem. Sect. B* **1977**, *15B*, 919–921.

[24] The 216-nm (ε 7200–7800) bands of **10** and **11** are like that of the conjugated lactone 5α,5β,17β-trihydroxy-3,5-seco-*A*-norandrost-1-en-3-oic acid lactone (3→5β) at 217 nm (ε 8000) [cf.: Caspi, E.; Khan, B. T.; Balasubrahmanyam, S. N. *Tetrahedron* **1962**, *18*, 1013–1018] but different from the alternative possibility of cyclic enol lactone such as 3-hydroxy-2,3-seco-*A*-norcholest-3-en-2-oic acid lactone (2→3) with absorption at 221.5 nm (log ε 3.69) [cf.: Heckendorn, R.; Tamm, Ch. *Helv. Chim. Acta* **1967**, *50*, 1499–1509].

[25] Steroid α,β-unsaturated lactones are characterized by lactone carbonyl, carbinyl, α-carbon, and β-carbon ^{13}C signals, respectively: γ-lactones, 171.1–176.8, 72.1–76.8, 116.2–121.3, and 167.6–177.2 ppm [cf.: (a) Tori, K.; Ishi, H.; Wolkowski, Z. W.; Chachaty, C.; Sangaré, M.; Piriou, F.; Lukacs, G. *Tetrahedron Lett.* **1973**, 1077–1080. (b) Tori, K.; Thang, T. T.; Sangaré, M.; Lukacs, G. *Ibid.* **1977**, 717–720. (c) Yamauchi, T.; Abe, F.; Nishi, M. *Chem. Pharm. Bull.* **1978**, *26*, 2894–2896. (d) Cruz, A.; Guzmãn, A.; Iriarte, J.; Medina, R.; Muchowski, J. M.; Massox, M. L. *J. Org. Chem.* **1979**, *44*, 3511–3515. (e) Cheung, H. T. A.; Coomb, R. G.; Sidwell, W. T. L.; Watson, T. R. *J. Chem. Soc., Perkin Trans. 1* **1981**, 64–72. (f) Brown, L.; Cheung, H. T. A.; Watson, T. R.; Nemorin, J. L. E. *Ibid.* **1981**, 1779–1781]; δ-lactones, 163.7–166.4, 79.6–84.0, 113.4–122.2, and 145.4–167.0 ppm [cf.: (g) Gasič, N. H.; Djarmati, Z.; Pelletier, S. W. *J. Org. Chem.* **1976**, *41*, 1219–1221. (h) Weihe, G. R.; McMorris, T. C. *Ibid.* **1978**, *43*, 3942–3946. (i) Eisner, T.; Weimer, D. F.; Haynes, L. W.; Meinwald, J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 905–908. (j) Meinwald, J.; Weimer, D. F.; Eisner, T. *J. Am. Chem. Soc.* **1979**, *101*, 3055–3060]. Saturated steroid lactones are characterized by carbonyl and carbinyl ^{13}C signals, respectively: δ-lactones, 163.9–174.8 and 83.1–86.3 ppm [cf. ref 25g–j]; ε-lactones, 176.2–178.5 and 63.3–70.5 ppm [cf.: (k) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D.; Stevens, G. L.; Flippen-Anderson, J. L.; Cook, J. C. *Nature (London)* **1979**, *281*, 216–217. (l) Thompson, M. J.; Mandava, N.; Flippen-Anderson, J. L.; Worley, J. F.; Dutky, S. R.; Robbins, W. E.; Lusby, W. L. *J. Org. Chem.* **1979**, *44*, 5002–5004. (m) Dave, V.; Strothers, J. B.; Warnhoff, E. W.; *Can. J. Chem.* **1980**, *58*, 2666–2678. (n) Wada, K.; Marumo, S. *Agric. Biol. Chem.* **1981**, *45*, 2579–2585].

and **12**. Additionally, acetate carbonyl signals at 168.91–171.84 ppm in ^{13}C spectra were observed.

Finally, bands at 1730–1745 cm^{-1} (overlapping aldehyde absorptions of **8** and **8** 3β-acetate) were recognized as probably arising from δ- or ϵ-lactone features in **8**, **8** 3β-acetate, and **12**. Carbonyl and carbinyl ^{13}C resonances at 169.45–169.48 and 87.06–87.47 ppm, respectively, confirmed the matter.[25] Moreover, the α,β-unsaturated carbonyl derivatives **10** and **11** were recognized as being α,β-unsaturated lactones analogous to **8** 3β-acetate and **12**. Carbonyl and carbinyl ^{13}C signals at 165.99 and 85.40 ppm, respectively, in the ^{13}C spectrum of **11** confirmed the formulation.

The aldehyde, acetate ester, and lactone features thereby established exhaust the oxygen functionality of **8** 3β-acetate, **10**, and **11** but not of the most highly oxygenated product **12**, which has yet one other oxygen atom to define. A three-proton signal at 3.30 ppm and ^{13}C signal at 55.94 ppm identified this final oxygen atom as part of a methoxyl group.

These structural features were also supported by mass spectra, which included a series of elimination ions $(M - H_2O)^+$, $(M - CH_3CO_2H)^+$, $(M - CH_3OH)^+$, and $(M - CO_2)^+$[26] and multiple elimination ions $(M - H_2O - CH_3CO_2H)^+$, $(M - 2CH_3CO_2H)^+$, $(M - CH_3OH - CH_3CO_2H)^+$, and $(M - CH_3OH - 2CH_3CO_2H)^+$ appropriate to each disecolactone. In addition to elimination ions supporting the assigned functionalities, fragment ions served to locate all oxygen functions in the A/B-ring system for all 5,6-secosterols. A prominent ion *m/z* 247 in spectra of peroxides **6** and their 3β-lacetates, secoaldehyde **3**, secotriol **5**, and lactones **8** 3β-acetate, **10**, and **12** as well as of other 5,6-secosterols[27] and of other cholestane derivatives with divers A/B-ring features[26b,d,e,27,28] was recognized as being the fragment ion $(C_{18}H_{31})^+$ derived by scission of the C-7/C-8 and C-9/C-10 bonds and representing the C/D-ring system (less one hydrogen) with the intact sterol side chain.

Furthermore, the fragment ion *m/z* 125 prominent in spectra of **8** 3β-acetate and **10–12** was recognized as being $(C_7H_9O_2)^+$, representing the intact A ring of disecolactones **10** and **11**, derived by scission of the C-9/C-10 bond (with ester elimination).[29]

These data establish that **8** 3β-acetate and **10–12** be 5,6-secosterol A-ring lactones derived by insertion of an oxygen atom into the C-5/C-10 bond geminal to the original 5ξ,6ξ-epidioxide feature of the parent peroxides **6**.[30] The predominant product **8** 3β-acetate is thus the 3β-acetate of **8** derived spontaneously from **6a**. Product **10** is clearly the Δ^3-analogue or elimination product of **8** or **8** 3β-acetate.

Structures of lactone esters **11** and **12** are also apparent from spectra. Lack of aldehyde feature in **11** but the presence of two acetoxyl groups (evinced by ^{13}C and mass spectra) suggest a hydrated 6-aldehyde diacetate moiety that is confirmed by a unique one-proton triplet signal at 6.96 ppm clearly deshielded by double oxygen substitution. Accordingly, **11** is 6,6-diacetoxy-10-hydroxy-5,6:5,10-disecocholest-3-en-5-oic acid lactone (5→10).

Lactone **12** is also a diacetate but a 5.12 ppm signal typical of the 3α-proton of sterol 3β-acetates demonstrated that the two acetoxyl groups were at different sites. Like **11**, a 6-aldehyde is absent in **12**, and a deshielded one-proton signal at 5.87 ppm established double oxygen substitution at C-6. Lactone **12** is thus 3β,6ξ-diacetoxy-10-hydroxy-6ξ-methoxy-5,6:5,10-disecocholestan-5-oic acid lactone (5→10). The assigned structure is further supported by the presence of a two-proton ABX signal of the 4-methylene protons at 3.00 ppm that collapsed to an AB doublet upon decoupling from the vicinal 3α-hydrogen.

The A-ring conformation of **8**, **8** 3β-acetate, and **12** appears to be a mobile chair, as the 3α-hydrogen signal half-width is 10.3 Hz, thus intermediate between that of axial and equatorial protons. Although the C-10 stereochemistry is not addressed by our data, inversion of the migrating carbon atom is not observed in related Baeyer–Villiger-type oxygen insertion reactions,[31] and we assume that lactones **8**, **8** 3β-acetate, and **10–12** retain the C-10 stereochemistry of parent cholesterol, thus a 10β-methyl group and 10*R* configuration.

Discussion

Our results confirm that the oxidation of cholesterol by O_3 yields 5,6-secosterols as major products, poorly characterized peroxidic secosterols **2a** in nonparticipating solvents, peroxides **6** in participating hydroxylic media. Moreover, peroxides **6a** and **6b** do not derive from **2a** (prepared in nonparticipating solvents) treated with water or methanol, respectively. A similar failure of ethanol to transform ozonide **2b** preparations to **6c** 3β-acetate had been previously noted.[4e] Accordingly, peroxides **6** appear to form by reaction of hydroxylic solvent with an unisolated and undetected ozonization intermediate such as a Criegee zwitterionic carbonyl oxide.[11,32] Location of the alkoxyl groups in **6b**, **6c**, and **6d** at C-6 by our spectral data then infers the 5-oxo-6-carbonyl oxide structure **9** for the putative Criegee zwitterion implicated in these experiments and eliminates the isomeric 6-oxo-5-carbonyl oxide possibility.

Furthermore, our results establish a second minor mode of oxidative attack of O_3 on cholesterol in polar media, one in which the isomeric 5,6-epoxides **7** are products. Cholesterol epoxidation by O_3 at dry ice temperatures did not occur in less polar aprotic solvents or in such media containing alcohols but was observed in ethyl acetate solutions and in alcoholic media at room temperature. Thus, epoxidation if favored by increased solvent polarity in a complex manner, in general agreement with the previously recognized dependence of olefin epoxidation on medium polarity and the thesis that olefin epoxidation by O_3 proceed by a mechanism different from that implicated in carbon–carbon bond scission.[11,32]

The two other ozonization products **3** and **8** recovered from aqueous systems in which **6a** formed are clearly subsequent

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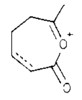
(26) Mass spectra of a variety of steroid lactones include $(M - CO)^+$, $(M - CO_2)^+$, both ions, or neither, as well as $(M - H_2O)^+$ ions; cf.: (a) ref 23b. (b) Budzikiewciz, H.; Buchler, J.; Quinkert, G. *Monatsh. Chem.* **1967**, *98*, 1115–1127. (c) Genard, P.; Palem-Vliers, M.; Coninx, P.; Margoulies, M.; Compernolle, F.; Vandewalle, M. *Steroids* **1968**, *12*, 763–776. (d) Ahmad, M. S.; Mushfiq, M.; Asif, M.; Ansari, G. A. S. *J. Prakt. Chem.* **1975**, *317*, 1049–1053. (e) Ahmad, M. S.; Moinuddin, G.; Khan, I. A. *Org. Mass Spectrom.* **1978**, *13*, 382–385. (f) Dias, J. R.; Ramachandra, R.; Nassim, B. *Ibid.* **1978**, *13*, 307–314.

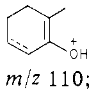
(27) Ahmad, M. S.; Mushfiq, M.; Ansari, G. A. S.; Waris, F. *Org. Mass Spectrom.* **1974**, *8*, 1–10.

(28) (a) Knights, B. A. *J. Gas Chromatogr.* **1967**, *5*, 273–282. (b) Templeton, J. F.; Wie, C. W. *Can. J. Chem.* **1974**, *52*, 517–523. (c) Knapp, F. F.; Wilson, M. S.; Schroepfer, G. J. *Chem. Phys. Lipids* **1976**, *16*, 31–50. (d) Knapp, F. F.; Schroepfer, G. J. *Ibid.* **1976**, *17*, 466–500. (e) Wyllie, S. G.; Amos, B. A.; Tökés, L. *J. Org. Chem.* **1977**, *42*, 725–732. (f) Partridge, L. F.; Djerassi, C. *Ibid.* **1977**, *42*, 2799–2805. (g) Turecek, F.; Kahout, L. *Collect. Czech. Chem. Commun.* **1980**, *45*, 2433–2441.

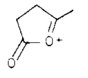
(29) Ion *m/z* 125 (Calcd for $C_7H_9O_2$: M, 125.06024. Found: M, 125.06000) of structure iv (also found in spectra of **6** and **6b** and **6c** 3β-



iv, *m/z* 125; Δ^3
v, *m/z* 127



vi, *m/z* 110; Δ^3
vii, *m/z* 112



viii, *m/z* 99

acetate) is a Δ^3-analogue of the fragment ion *m/z* 127 $(C_7H_{11}O_2)^+$ of structure v, which represents the A-ring features of 5,6;5,10-disecocholestane-5,6-dioic acid lactone (5→10) 6-methyl ester; cf. ref 23c. Ions *m/z* 110 $(C_7H_{10}O)^+$ and *m/z* 112 $(C_7H_{12}O)^+$ of structures vi and vii, respectively, were derived from several related 5,6-seco-5-ketones and Δ^3-5,6-seco-5-ketones; cf. ref 27. Moreover, ion *m/z* 110 is also prominent in spectra of **6a–c** and secoaldehyde **3**. Ion *m/z* 99 $(C_5H_7O_2)^+$ of structure viii was similarly derived from 3,5;-5,10-diseco-*A*-norcholestane-3,6-dioic acid lactone (3→10) 6-methyl ester; cf. ref 23b.

(30) Alternative formulations involving oxygen insertions into the C-4/C-5 bond to give 4,5;5,6-disecolactones do not account for the significantly deshielded C-19 proton signals of **8**, **8** 3β-acetate, and **10–12** and are not consistent with other spectral details. Moreover, α,β-unsaturated 4,5;5,6-disecolactones cannot form, nor would alternative A-ring enolic lactone formulations be consistent with spectral data.

(31) (a) Hassall, C. H. *Org. React.* (*N.Y.*) **1957**, *9*, 73–106. (b) Kirk, D. N.; Hartshorn, M. P. "Steroid Reaction Mechanisms"; Elsevier: Amsterdam/London/New York, 1968; pp 345–349.

(32) (a) Murray, R. W. *Acc. Chem. Res.* **1968**, *1*, 313–333. (b) Criegee, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 745–752.

transformation products of **6a** and are not initially formed products of the attack of O_3 on cholesterol but may be regarded as arising from **6a** via processes induced thermally or by protonation, with subsequent loss of the elements of H_2O_2 to give secoaldehyde **3** or of water (with oxygen insertion) to yield **8**.

The instability of peroxides **6** is further evinced by their observed rearrangement upon treatment with acetic anhydride/pyridine. Similar oxygen insertion reactions have been reported for steroid tertiary hydroperoxides vicinal to carbonyl or olefinic substitution, steroid 17α-hydroperoxy-20-ketones undergoing β-scission but also rearrangement to *D-homo*-17a-oxasteroids, 5-hydroperoxy-5α-estr-6-en-17-one rearranging to *B-homo*-6-oxaestra-4,7-dien-17-one.[33]

The transformations of peroxides **6** to disecolactones **8**, **8** 3β-acetate, and **10–12** are reminiscent of the related Baeyer–Villiger oxidation of steroid ketones by peracids or peroxides.[31] Indeed, peroxides **6** are cyclic analogues of the putative Baeyer–Villiger peroxy alcohol intermediate from which oxygen insertion proceeds. Furthermore, these rearrangements of peroxides **6** to disecolactones involved regiospecific insertion into the C-5/C-10 bond, thus exactly like the reported Baeyer–Villiger oxidation of methyl 5-oxo-5,6-secocholestan-6-oate, which gave only one rearranged product 10-hydroxy-5,6:5,10-disecocholestane-5,6-dioic acid lactone (5→10) 6-methyl ester.[23c]

By analogy to the Baeyer–Villiger mechanism, rearrangements of **6** are viewed as resulting from initial protonation of the 5ξ,6ξ-epidioxide C-5 oxygen atom, followed by migration of the C-10 carbon atom to the resultant C-5 oxonium cation, yielding postulated intermediates 3β,6,6,10-tetrahydroxy-5,6;5,10-disecocholestan-5-oic acid lactone (5→10) (**13a**) from **6a**, 3β,6ξ,10-trihydroxy-6ξ-methoxy-5,6;5,10-disecocholestan-5-oic acid lactone (5→10) (**13b**) from **6b**. Subsequent elimination of the elements of water or alcohol (and 3β-acetylation) from **13** would then give the predominant product **8** 3β-lactate, with **10** formed by eliminations from **8** or **8** 3β-acetate.

Products **11** and **12** retaining the doubly oxygenated C-6 carbon atom of parents **6a** and **6b** then arise by competing acetylations of **13**, **11** from **13a** via postulated intermediate 3β,6,6-triacetoxy-5,6;5,10-disecocholestan-5-oic acid lactone (5→10) (**14**) and **12** from **13b** directly.

Our present results bear on the current problem of the origins of "anomalous" ozonization products derived via oxygen insertion reactions during or subsequent to attack of O_3 on olefins, as lactones **8** and **10–12** are formally "anomalous" cholesterol ozonization products. A sound case has been built for rearrangement of initially formed ozonides as origin of such "anomalous" lactones,[11b,34] but other evidence suggests derivation via rearrangement of postulated Criegee zwitterionic carbonyl oxides to dioxirane intermediates with subsequent insertion of dioxirane oxygen leading to lactone products.[32,35]

For such a process to account for products **8** and **10–12**, there must be preceding dissociations of the parent peroxides **6**, not to 3β-hydroxy (or acetoxy)-5-oxo-5,6-secocholestane-6-carbonyl oxide (**9**) implicated in the derivation of **6** from cholesterol (or **1b**) but to the isomeric 3β-hydroxy (or acetoxy)-6-oxo-5,6-secocholestane-5-carbonyl oxide necessary for rearrangement to the dioxirane required for oxygen insertion into the C-5/C-10 bond. However, products **11** and **12** could not derive from such an isomeric carbonyl oxide, as a 6-aldehyde hydrate, methyl hemiacetal, or other unprecedented feature would then be required for their derivation.

On balance, our results more satisfactorily support the thesis that "anomalous" lactone products of ozonization derive via rearrangement of initially formed ozonide or epidioxide.[36]

Experimental Section[37]

Aqueous dispersions of pure cholesterol freed from detectable autoxidation products were made by dissolving 100 mg of cholesterol in 50 mL of acetone, adding the solution to 120 mL of distilled water in a rotary evaporator under vacuum, and evaporating the dispersion to remove solvent and some water and provide a stable 1 mg/mL cholesterol dispersion, which was filtered through sintered glass and used as such. Solutions of cholesterol (1 mg/mL) were made in specified neat organic solvents; solutions in aqueous organic solvents were made by adding neat solvent solutions to an equal volume of water.

Ozone was generated with a Tesla coil leak detector (Micro-Ozonizer, Supelco Inc., Bellefonte, PA) discharge into a stream of O_2 flowing at 1 L/min. The O_2–O_3 stream (containing approximately 0.18 mequiv of O_3/min) was passed through the solution or dispersion of cholestrol to be oxidized, with aliquots being analyzed by thin-layer chromatography periodically to monitor the course of the reaction.

5ξ,6ξ-Epidioxy-5,6-secocholestane-3β,5ξ,6ξ-triol (6a). A. From Water Dispersions. A 1 mg/mL aqueous dispersion of cholesterol (104 mg) was ozonized at room temperature for 2 h, after which time the dispersion was saturated with solid NaCl and extracted with benzene. The benzene extracts were dried over anhydrous Na_2SO_4, and solvent was removed under vacuum (without heating) to give white solids containing **3**, **6a**, **7**, and **8**. Chromatography on silica gel irrigated with hexane–diethyl ether (3:2) or methylene chloride–diethyl ether (7:3) resolved the components, the most polar fractions containing **6a**, recovered upon evaporation of solvent under vacuum without heat. Recrystallization of **6a** gave 45 mg (38.5%): mp 116–117.5 °C; IR (KBr) 3200–3600 (OH), 1145, 1022, 1010, 990, 958 cm^{-1}; ^1H NMR 0.67 (3 H, s, C-18), 1.06 (3 H, s, C-19), 2.66 (1 H, dd, J = 3.8, 12.8 Hz, 4α-H), 3.88 (1 H, m, $W_{1/2}$ 26 Hz, 3α-H), 5.22 ppm (1 H, t, J = 7.7 Hz, 6ξ-H); EI mas spectrum, m/z (%) 434 (0.4) $(M - H_2O)^+$, 419 (0.7), 416 (1.6) $(M - 2H_2O)^+$, 398 (0.7) $(M - 3H_2O)^+$, 372 (0.6), 303 (2.0), 285 (1.3), 262 (7.5), 247 (7.3), 245 (3.3), 171 (6.3), 143 (100), 135 (23.3), 128 (25.3), 125 (10.0), 110 (20.0); CI mass spectrum, m/z (%) 453 (44) $(M + H)^+$, 435 (100) $(M - H_2O + H)^+$, 417 (78) $(M - 2H_2O + H)^+$, 399 (17) $(M - 3H_2O + H)^+$; R_f 0.26 (system I), 0.14 (system II); t_R 28.0 min (μPorasil); decomposition on gas chromatography. Anal. Calcd for $C_{27}H_{48}O_5$: $M - H_2O$, 434.33959;

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(33) (a) Gardner, J. N.; Carlon, F. E.; Gnoj, O. *J. Org. Chem.* **1968**, *33*, 1566–1570. (b) Peters, J. A. M.; van Vliet, N. P.; Zeelan, F.J. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 226–228.

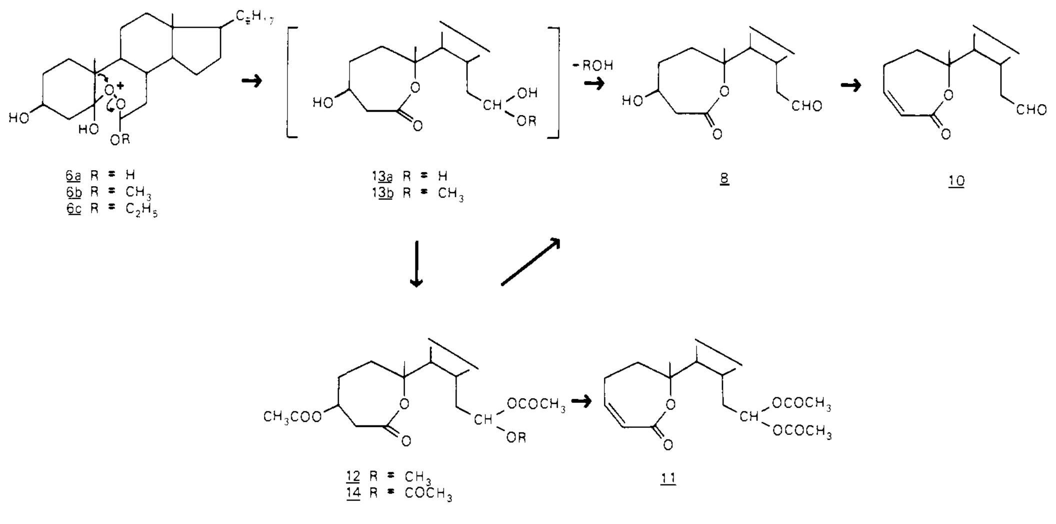
(34) (a) Nahavandi, F.; Razmara, F.; Stevens, M. P. *Tetrahedron Lett.* **1973**, 301–304. (b) Howard, J. A.; Mendenhall, G. D. *Can. J. Chem.* **1975**, *53*, 2199–2201. (c) Rio, G.; Scholl, M.-J. *J. Chem. Soc., Chem. Commun.* **1975**, 474. (d) Feringa, B. L.; Butselear, R. J. *Tetrahedron Lett.* **1981**, *22*, 1447–1450. (e) Saito, I.; Nakata, A.; Matsuura, T. *Ibid.* **1981**, *22*, 1697–1700.

(35) (a) Yamamoto, Y.; Niki, E.; Kamiya, Y. *J. Org. Chem.* **1981**, *46*, 250–254. (b) Adam, W.; Rodriguez, A. *Tetrahedron Lett.* **1981**, *22*, 3509–3512.

(36) Unidentified more highly oxidized products have been isolated from ozonizations of **1b**, nonperoxidic products $C_{29}H_{48}O_4$ (mp 197–198 °C) and $C_{29}H_{48}O_6$ (mp 126 °C, cf. ref 4e), and peroxidic products $C_{29}H_{48}O_{4.5}$ (mp 221–223 °C) and $C_{29}H_{48}O_4$ (mp 235–236 °C, vf. ref 4f). None of these correspond to disecolactones **8** 3β-acetate or **10–12**.

(37) Melting points were taken on a Kofler block under a microscope. Thin-layer chromatography was conducted with 10-cm long Alugram Sil G/UV_{254} aluminum-backed chromatostrips (Machery Nagel, Düren) irrigated with system I, benzene–ethyl acetate (3:2); system II, benzene–ethyl acetate (7:3); system II, benzene–ethyl acetate (4:1); or system IV, benzene–ethyl acetate (9:1). Sterols were detected by examining under 254-nm light by using *N*,*N*-dimethyl-*p*-phenylenediamine spray for peroxides **6** and **6b** and **6c** 3β-acetate (cf.: Smith, L. L.; Hill, F. L. *J. Chromatogr.* **1972**, *66*, 101–109) and 50% sulfuric acid spray with heating to obtain brown colors for all secosterols. High-performance liquid column chromatography was conducted with two 3.9 mm × 30 cm μPorasil microparticulate (10-μm diameter) adsorption columns in tandem (Waters Associates, Milford, MA) irrigated with hexane–isopropyl alcohol (24:1) flowing at 2.0 mL/min for compounds **1–8**, at 1.0 mL/min for disecolactones **8** 3β-acetate and **10–12** (cf.: Ansari, G. A. S.; Smith, L. L. *Ibid.* **1979**, *175*, 307–315). Effluent was monitored by absorption at 212 nm measured with a Perkin-Elmer Model LC-55 variable wavelength spectrophotometric detector and by differential refractive index by using a Waters Associates Model R4-1 detector. Gas chromatography was conducted on fused silica capillary columns 0.2-mm i.d., 25-m long, wall-coated with SE-30 (Applied Science, State College, PA) and a Finnigan Corp. Model 3300 gas chromatograph–mass spectrometer (He carrier gas). Injector and detector were held at 285 °C; oven temperature was programmed from 100 °C (held for 1 min) to 270 °C at 20 °C/min. Mass spectra were recorded in EI mode at 15 eV for peroxides **6** and **6b** and **6c** 3β-acetate and at 72 eV for secosterols **3–5** and in the CI mode (only ions above m/z 200 considered) with methane as reagent gas on Finnigan Corp. Models 3200, 3300, and 4000 quadrupole mass spectrometers. High-resolution mass measurements were made with a CEC 21-110B instrument at 30 eV. Fourier transform proton (90-MHz) and proton- and off-resonance-decoupled ^{13}C (22.63-MHz) spectra were recorded on deuteriochloroform solutions with a JEOL Model FX90Q spectrometer (Department of Chemistry, William Marsh Rice University, Houston, TX). Ultraviolet light absorption measurements were made on ethanol solutions of sterols with a Cary Model 14 spectrophotometer. Infrared absorption spectra over the range 400–4000 cm^{-1} were recorded on CCl_4 solutions of sterol or on 0.1-mm diameter KBr pellets with a Perkin-Elmer Model 337 infrared spectrometer equipped with a beam condenser.

Chart II



M − $2H_2O$, 416.32903. Found: M − H_2O, 434.3389; M − $2H_2O$, 416.3296.[38]

B. From Aqueous Tetrahydrofuran. A solution of 100 mg of cholesterol in 100 mL of tetrahydrofuran–water (1:1) was ozonized at room temperature for 1.5 h and processed according to (A) above. Thus was recovered 47.6 mg (40.7%) of peroxide **6a**: (mp 116–117 °C), 39.7 mg (33.9%) of secoaldehyde **3**, and 16.8 mg of 5,6-epoxides **7** (one-tenth of which was individually resolved on a semipreparative μPorasil column to give 0.187 mg (1.8%) of 5α,6α-epoxide **7a** and 1.489 mg (14.3%) of 5β,6β-epoxide **7b**), all identified by combinations of spectral and chromatographic data in comparisons with authentic reference samples.

3β-Hydroxy-5-oxo-5,6-secocholestan-6-al (3). A. From Aqueous Dispersions. Material eluted with hexane–diethyl ether (17:1) and (17:3) from silica gel columns from which **6a** was recovered gave secoaldehyde **3** that was rechromatographed on silica gel to afford pure **3** as a colorless oil:[16] 38.6 mg (35.7%); IR (CCl_4) 3650 and 3450 (OH), 2730 (CHO), 1730 (CHO), 1710 cm^{-1} (CO) (lit. IR (Nujol) 2688, 1739, 1704 cm^{-1} [4f]); ^{1}H NMR 0.68 (3 H, s, C-18), 1.02 (3 H, s, C-19), 3.12 (1 H, dd, J = 3.8, 14.1 Hz, 4α-H), 4.48 (1 H, m, $W_{1/2}$ 7.7 Hz, 3α-H), 9.62 ppm (1 H, s, $W_{1/2}$ 4 Hz, 6ξ-H); EI mass spectrum, m/z (%) 418 (4.6) (M)$^+$, 400 (45.0) (M − H_2O)$^+$, 376 (12.2), 382 (32.0) (M − $2H_2O$)$^+$, 372 (100), 358 (39.3), 354 (37.8) 318 (24.4), 314 (12.9), 291 (11.5), 249 (25.9), 247 (48.8), 149 (45.0), 110 (68.7); CI mass spectrum, m/z (%) 419 (14) (M + H)$^+$, 401 (36) (M − H_2O + H)$^+$, 399 (32), 389 (36), 383 (100) (M − $2H_2O$ + H)$^+$, 381 (36), 372 (55), 370 (46), 368 (50), 366 (46), 356 (82), 355 (27), 354 (46); R_f 0.49 (system I), 0.37 (system II); t_R 17.2 min (μPorasil), t_R 24.0 min (SE-30).

B. From Ozonide Reduction. Crude solids (50 mg) obtained upon evaporation under vacuum of benzene extracts of ozonized aqueous dispersions of cholesterol were dissolved in 10 mL of acetic acid, and 50 mg of Zn dust was added. The mixture was occasionally shaken and was allowed to stand overnight. Following benzene extraction, washing of extracts with $NaHCO_3$ solution and with water, and drying over anhydrous Na_2SO_2, there was recovered a mixture of **3**, **7a**, and **7b** (**3** predominant). Chromatography of one-tenth of the material on a semipreparative μPorasil column irrigated with hexane–isopropyl alcohol (24:1) at 2.0 mL/min gave 8.35 mg (77.2%) of **3**, 0.19 mg (1.8%) of **7a**, and 1.53 mg (14.7%) of **7b**, all identified by chromatographic and spectral data with references to authentic samples.

5,6α-Epoxy-5α-cholestan-3β-ol (7a) and 5,6-Epoxy-5β-cholestan-3β-ol (7b). A middle fraction (21.48 mg) eluted from the silica gel column from which **3** and **6a** were recovered consisted of a 1:8 mixture of 5,6-epoxides **7**. Rechromatography of one-tenth of the fraction on μPorasil irrigated with hexane–isopropyl alcohol (24:1) gave 0.198 mg of 5α,6α-epoxide **7a** [mp 137–139 °C; R_f 0.44 (system I), 0.33 (system II); t_R 13.0 min (μPorasil), t_R 27.1 min (SE-30)] and 1.52 mg (14.6%) of 5β,6β-epoxide **7b** [mp 130–132 °C; R_f 0.45 (system I), 0.35 (system II); t_R 14.0 min (μPorasil), t_R 26.6 min (SE-30)], all identical in these properties and in EI mass spectra with authentic reference samples.

3β,10-Dihydroxy-6-oxo-5,6;5,10-disecocholestan-5-oic Acid Lactone (5→10) (8). Following elution of **6a** as the most polar of the prominent products of cholesterol ozonization in water, there was eluted a small and variable amount of a fifth oxidation product **8** as a colorless oil: IR (CCl_4) 3450 (OH), 2730 (CHO), 1730 cm^{-1} (CO); ^{1}H NMR 0.68 (3 H, s, C-18), 1.37 (3 H, s, C-19), 2.96 (1 H, d, J = 3 Hz, 4-H), 4.17 (1 H, m, $W_{1/2}$ 10.3 Hz, 3α-H), 9.70 ppm (1 H, s, CHO); CI mass spectrum, m/z (%) 435 (52) (M + H)$^+$, 417 (100) (M − H_2O + H)$^+$, 399 (6) (M − $2H_2O$ + H)$^+$; R_f 0.30 (system I), 0.16 (system II).

5ξ,6ξ-Epidioxy-6ξ-methoxy-5,6-secocholestane-3β,5ξ-diol (6b). A. From Aqueous Methanol. A solution of 100 mg of cholesterol in 100 mL of methanol–water (1:1) was ozonized at room temperature for 30 min. Precipitated crystals were filtered, yielding 85 mg (70.9%) of chromatographically pure **6b**, which was recrystallized twice from acetone: mp 137–139 °C (lit.[4e] mp 139–140 °C); IR (KBr) 3320, 1145, 1070, 1045, 1025, 960, 935, 920 cm^{-1}; IR (CCl_4) 3340, 1150, 1070, 1045, 1020, 985, 960, 935, 920 cm^{-1} (lit. IR (KBr) 3320 cm^{-1}, IR (CCl_4) 3340 cm^{-1} [4e]); ^{1}H NMR 0.67 (3 H, s, C-18), 1.08 (3 H, s, C-19), 2.66 (1 H, dd, J = 3.8, 13.4 Hz, 4α-H), 3.50 (3 H, s, OCH_3), 3.88 (1 H, m, $W_{1/2}$ 26 Hz, 3α-H), 4.62 ppm (1 H, t, J = 7.7 Hz, 6ξ-H); ^{13}C NMR 55.7 (OCH_3), 66.9 (C-3), 102.6 (C-6), 112.0 ppm (C-5); EI mass spectrum, m/z (%) 448 (1.2) (M − H_2O)$^+$, 434 (1) (M − CH_3OH)$^+$, 433 (1.2), 417 (6.7), 416 (3.0) (M − H_2O − CH_3OH)$^+$, 399 (4.1), 398 (1.2) (M − $2H_2O$ − CH_3OH)$^+$, 372 (2.5), 360 (6.9), 331 (7.1), 328 (11.2), 287 (4.0), 285 (3.3), 274 (10.6), 263 (64.0), 247 (19.3), 185 (20.0), 143 (100), 135 (37.3), 128 (42.7), 125 (8.0), 110 (13.3), 109 (16.0); CI mass spectrum, m/z (%) 467 (1) (M + H)$^+$, 458 (2), 449 (7) (M − H_2O + H)$^+$, 447 (5), 445 (8), 435 (12), (M − CH_3OH + H)$^+$, 433 (17) (M − CH_3OH − H)$^+$, 417 (100) (M − H_2O − CH_3OH + H)$^+$, 399 (32) (M − $2H_2O$ − CH_3OH + H)$^+$; R_f 0.58 (system I), 0.41 (system III); t_R 19.33 min (μPorasil).

Chromatography on silica gel of the acetone mother liquors yielded an additional 5.54 mg of **6b** (total yield 75.0%) and 15.1 mg (14.5%) of 5,6-epoxides **7**, one-tenth of which was chromatographed on μPorasil with hexane–isopropyl alcohol (24:1) to give 0.166 mg (1.6%) of 5α,6α-epoxide **7a** and 1.343 mg (12.9%) of 5β,6β-epoxide **7b**, both identified by spectral and chromatographic data in comparison with reference samples.

B. From Chloroform–Methanol. A solution of 0.5 g of cholesterol in 100 mL of chloroform–methanol (1:1) was ozonized at dry ice temperature for 15 min. After removal of solvent and crystallization from methanol, there was recovered 573 mg (90.9%) of **6b**, identified by spectral and chromatographic properties with **6b** prepared in aqueous methanol.

3β-Acetoxy-5ξ,6ξ-epidioxy-6ξ-methoxy-5,6-secocholestan-5ξ-ol. A solution of 100 mg of cholesterol 3β-acetate in 20 mL of chloroform–methanol (1:1) ozonized at dry ice temperature for 15 min gave, after removal of solvent under vacuum and chromatography on μPorasil, 105.3 mg (88.7%) of **6b** 3β-acetate: mp 143–145 °C (from methanol) (lit. mp 145–146 °C,[4e] 151–152 °C dec[8]); IR (KBr) 3450, 3320, 1740, 1255, 1150, 1060, 1050, 1030, 1015, 995, 960, 940, 925 cm^{-1}; IR (CCl_4) 3330, 1750, 1250, 1150, 1062, 1048, 1028, 1018, 995, 963, 940, 920 cm^{-1} (lit. IR (Nujol) 3250, 1740, 1250 cm^{-1}, IR (KBr) 3320, 1742, 1243 cm^{-1} [8]); ^{1}H NMR 0.64 (3 H, s, C-18), 1.06 (3 H, s, C-19), 2.02 (3 H, s, CH_3CO), 2.68 (1 H, dd, J = 3.8, 13.4, 4α-H), 3.46 (3 H, s, OCH_3), 4.60 (1 H, t, J = 7.7 Hz, 6ξ-H), 4.89 ppm (1 H, m, $W_{1/2}$ 22 Hz, 3α-H); ^{13}C NMR 21.3 (CO*C*H$_3$), 55.8 (OCH_3), 69.8 (C-3), 102.6 (C-6), 111.7 (C-5), 171.1 ppm (*C*OCH$_3$); EI mass spectrum, m/z (%) 508 (0.1) (M)$^+$, 490 (2.2) (M − H_2O)$^+$, 476 (0.4), 459 (4.4), 458 (2.9) (M − H_2O − CH_3OH)$^+$, 430 (2.9) (M − H_2O − CH_3CO_2H)$^+$, 416 (2.5), 339 (29.4), 398 (8.8) (M − H_2O − CH_3OH − CH_3CO_2H)$^+$, 285 (14.7), 274 (18.6), 263 (100), 247 (31.8), 203 (38.8), 185 (6.6), 143 (72.9), 135 (62.3), 125 (9.3), 110 (61.8); CI mass spectrum, m/z (%) 491 (9) (M − H_2O + H)$^+$, 489 (11) (M − H_2O − H)$^+$, 476 (6), 474 (5), 495 (56) (M − H_2O −

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[38] A molecular ion was not found.

$CH_3OH + H)^+$, 477 (17), 431 $(M - H_2O - CH_3CO_2H + H)^+$, 417 (49), 339 (100) $(M - H_2O - CH_3OH - CH_3CO_2H + H)^+$.

5ξ,6ξ-Epidioxy-6ξ-ethoxy-5,6-secocholestane-3β,5ξ-diol (6c). A solution of 100 mg of cholesterol in 20 mL of chloroform (stabilized with ethanol) was ozonized for 15 min at dry ice temperature. After removal of solvent, one-onehundredth of the crude product was chromatographed on μPorasil irrigated with hexane–isopropyl alcohol (24:1), thereby yielding 1.122 mg (92.0%) of **6c**: mp 133–134 °C (lit. mp 137 °C[4e]); IR (KBr) 3250, 1150, 1075, 1050, 1020, 990, 955 cm^{-1}; IR (CCl_4) 3650, 3320, 1150, 1140, 1070, 1050, 1020, 995, 955 cm^{-1}; ^1H NMR 0.64 (3 H, s, C-18), 1.06 (3 H, s, C-19), 1.24 (3 H, t, $J = 7.7$ Hz, OCH_2CH_3), 2.64 (1 H, dd, $J = 3.8$, 14.1 Hz, 4α-H), 3.92 and 3.60 (2 H, ABX_3 m, OCH_2CH_3), 3.88 (1 H, m, $W_{1/2}$ 24 Hz, 3α-H), 4.72 ppm (1 H, t, $J = 7.7$ Hz, 6ξ-H); ^{13}C NMR 15.0 (CH_2CH_3), 64.0 (OCH_2), 66.9 (C-3), 100.8 (C-6), 115.8 ppm (C-5); EI mass spectrum, m/z (%) 462 (1.9) $(M - H_2O)^+$, 447 (1.3), 434 (2.4) $(M - C_2H_5OH)^+$, 416 (6.6) $(M - H_2O - C_2H_5OH)^+$, 401 (2.8), 398 (1.8) $(M - 2H_2O - C_2H_5OH)^+$, 345 (8.3), 303 (6.1), 285 (2.7), 277 (50.6), 249 (8.0), 247 (8.0), 199 (63.3), 143 (100), 135 (25.4), 128 (7.3), 125 (10.0), 110 (6.0), 109 (8.0); CI mass spectrum, m/z (%) 463 (6) $(M - H_2O + H)^+$, 445 (14) $(M - 2H_2O + H)^+$, 429 (14), 417 (67) $(M - H_2O - C_2H_5OH + H)^+$, 401 (49), 399 (56) $(M - 2H_2O - C_2H_5OH + H)^+$, 383 (100); R_f 0.62 (system I), 0.49 (system III); t_R 15.00 min (μPorasil).

3β-Acetoxy-5ξ,6ξ-epidioxy-6ξ-ethoxy-5,6-secocholestan-5ξ-ol. A solution of 100 mg of cholesterol 3β-acetate in 20 mL of chloroform (stabilized with ethanol) was ozonized at dry ice temperature for 15 min. Upon evaporation of solvent and crystallization from methanol, there was recovered 108.9 mg (89.3%) of **6c** 3β-acetate: mp 138–140 °C (lit. mp 140 °C[4e]); IR (KBr) 3430, 3300, 1740, 1250, 1150, 1060, 1030, 995, 960 cm^{-1}; IR (CCl_4) 3320, 1745, 1252, 1150, 1060, 1050, 1040, 1030, 995, 962 cm^{-1}; ^1H NMR 0.65 (3 H, s, C-18), 1.06 (3 H, s, C-19), 1.21 (3 H, t, $J = 7.7$ Hz, OCH_2CH_3), 2.02 (3 H, s, CH_3CO), 2.64 (1 H, dd, $J = 3.8$, 12.8 Hz, 4α-H), 3.61 and 3.87 (2 H, ABX_3 m, OCH_2CH_3), 4.71 (1 H, t, $J = 7.7$ Hz, 6ξ-H), 4.88 ppm (1 H, m, $W_{1/2}$ 24 Hz, 3α-H); ^{13}C NMR 15.1 (CH_2CH_3), 21.3 ($COCH_3$), 64.0 (OCH_2), 69.9 (C-3), 100.9 (C-6), 111.7 (C-5), 170.5 ppm ($COCH_3$); EI mass spectrum, m/z (%) 504 (4.0) $(M - H_2O)^+$, 476 (7.2) $(M - C_2H_5OH)^+$, 485 (5.2) $(M - H_2O - C_2H_5OH)^+$, 444 (4.0), 416 (12.9), 398 (20.0) $(M - H_2O - C_2H_5OH - CH_3CO_2H)^+$, 363 (5.0), 345 (16.0), 331 (5.3), 303 (5.0), 285 (12.7), 227 (100), 249 (50.6), 247 (12.0), 199 (6.3), 169 (22.7), 143 (24.0), 135 (28.0), 125 (16.0), 110 (12.0); CI mass spectrum, m/z (%) 523 (0.6) $(M + H)^+$, 521 (1) $(M - H)^+$, 507 (4), 506 (3), 505 (3) $(M - H_2O + H)^+$, 490 (1), 487 (2), 477 (3) $(M - C_2H_2OH + H)^+$, 476 (3) $(M - C_2H_5OH)^+$, 475 (3) $(M - C_2H_5OH - H)^+$, 459 (63) $(M - H_2O - C_2H_5OH + H)^+$, 399 (100) $(M - H_2O - C_2H_5OH - CH_3CO_2H + H)^+$.

5ξ,6ξ-Epidioxy-6ξ-*tert*-butoxy-5,6-secocholestane-3β,5ξ-diol (6d). A solution of 50 mg of cholesterol in 10 mL of *tert*-butyl alcohol was ozonized at room temperature for 15 min. Evaporation of solvent gave a solid crude product that was purified by chromatography on μPorasil irrigated with hexane–isopropyl alcohol (24:1) to yield 7.3 mg of 5,6-epoxides **7**, shown to be a 1:8 mixture of **7a** and **7b** by additional high-performance liquid chromatography and 47.83 mg (72.7%) of **6d** crystallized from ethanol: mp 134–136 °C; IR (KBr) 3450, 3280, 1175, 1145, 1090, 1068, 1025, 983, 925 cm^{-1}; IR (CCl_4) 3650, 3300, 1148, 1095, 1070, 1040, 1025, 1005, 990, 960 cm^{-1}; ^1H NMR 0.65 (3 H, s, C-18), 1.04 (3 H, s, C-19), 1.28 (9 H, s, $3CH_3$), 2.62 (1 H, ddd, $J = 1.8$, 4.0, 14.4 Hz, 4α-H), 3.88 (1 H, m, $W_{1/2}$ 25 Hz, 3α-H), 5.00 ppm (1 H, dd, $J = 6.6$, 10 Hz, 6ξ-H); ^{13}C NMR 28.5 ($C(CH_3)_3$), 44.5 ($C(CH_3)_3$), 67.0 (C-3), 94.7 (C-6), 111.7 ppm (C-5); EI mass spectrum, m/z (%) 490 (0.85) $(M - H_2O)^+$, 475 (2.0), 434 (20), 416 (26.6), 401 (12), 398 (8), 274 (8), 249 (32), 227 (32), 171 (29.3), 143 (100), 135 (57.3), 125 (17.3), 109 (22.6), 59 (42.6). Anal. Calcd for $C_{31}H_{56}O_5$: $M - H_2O$, 490.40219. Found: $M - H_2O$, 490.4028.[38]

5β-5,6-Secocholestane-3β,5α,6-triol (5). A. From Crude Ozonization Products. The crude ozonization products from 100 mg of cholesterol in aqueous dispersion were reduced with 120 mg of $NaBH_4$ overnight. Products were extracted with benzene, and the benzene extracts were dried over anhydrous Na_iSO_4, and evaporated under vacuum, and crystallized from acetone containing a few drops tetrahydrofuran. Thus was recovered 78.2 mg of crystalline secotriol **5**: mp 191–193 °C (lit. mp 192–193 °C[4e,6]); IR (KBr) 3600 cm^{-1}; EI mass spectrum, m/z (%) 422 (0.5) $(M)^+$, 404 (4.2) $(M - H_2O)^+$, 386 (10.3) $(M - 2H_2O)^+$, 356 (8.7), 348 (100), 332 (20.0), 330 (16.3), 319 (14.7), 304 (33.9), 303 (46.8), 301 (13.1), 293 (18.4), 291 (31.0), 247 (6.0), 191 (54.0); CI mass spectrum, m/z (%) 421 (7), $(M - H)^+$, 419 (5), 417 (4), 405 (100) $(M - H_2O + H)^+$, 403 (61) $(M - H_2O - H)^+$, 387 (82) $(M - 2H_2O + H)^+$, 385 (54) $(M - 2H_2O - H)^+$, 370 (64); R_f 0.02 (system I), 0.00 (system II), 0.25 (benzene–tetrahydrofuran (3:2)).

Chromatography on silica gel of the moter liquors gave an additional 3.8 mg of **5** (total yield 75.0%) and 16.7 mg (16.0%) of 5,6-epoxides **7**, shown to be a 1:8 mixture of **7a** and **7b** by capillary column gas chromatography on SE-30.

B. From Secoaldehyde 3. A solution of 20 mg of pure secoaldehyde **3** in 10 mL of tetrahydrofuran containing 5 mg of $LiAlH_4$ was refluxed for 15 min. Following isolation of sterol, there was obtained 18.0 mg (89.1%) of secotriol **5** (mp 191–193 °C) identical in spectral and chromatographic properties with the sample prepared in (A).

3β,6-Diacetoxy-5β-5,6-secocholestan-5α-ol. A solution of 50 mg of secotriol **5** in 5 mL of pyridine and 1 mL of acetic anhydride was kept for 48 h at room temperature. Following the usual processing, 50 mg of a chromatographically homogenous oily diacetate was obtained: IR (CCl_4) 3500, 1736, 1255, 1040 cm^{-1}; ^1H NMR 0.64 (3 H, s, C-18), 0.95 (3 H, S, C-19), 2.02 and 2.04 (6 H, s, CH_3CO), 4.04 (1 H, m, $W_{1/2}$ 11 Hz, 5β-H), 4.56 (2 H, AB q, $J = 15$ Hz, $6\text{-}CH_2$), 5.02 ppm (1 H, m, $W_{1/2}$ 26 Hz, 3α-H); EI mass spectrum, m/z (%) 446 (29.0) $(M - CH_3CO_2H)^+$, 428 (22.5) $(M - H_2O - CH_3CO_2H)^+$, 416 (38.1), 386 (19.3), 303 (100), 276 (25.8), 163 (51.6), 161 (32.2), 149 (51.6). Anal. Calcd for $C_{31}H_{54}O_5$: M, 506.3971. Found: M, 506.3957.

Ozonization of Cholesterol in Aprotic Organic Solvents. A solution of 50 mg of cholesterol in 5 mL of CCl_4 or methylene chloride was ozonized for 15 min at dry ice temperature. Removal of solvent under vacuum without heating gave 52 mg of solid crude ozonization products, all more mobile on thin-layer chromatograms irrigated with system IV than cholesterol. The mixture was chracterized by IR [IR (KBr) 3300, 1720, 1170, 1150, 1075, 1005, 995, 925 cm^{-1}; IR (CCl_4) 3320, 1720, 1170, 1150, 1070, 1015, 960, 922 cm^{-1}]. The 1720-cm^{-1} band increased in intensity after standing at room temperature for 48 h. Reduction of the crude products with Zn/acetic acid gave secoaldehyde **3** in 85% yield; reduction with $LiAlH_4$ gave 70% secotriol **5**, all products being identified by comparison of chromatographic and spectral data with those of authentic samples.

Acetylation Conditions. A solution of 50 mg of pure peroxide **6a** or **6b** or of **6b** 3β-acetate or **6c** 3β-acetate in 2.5 mL of pyridine and 0.5 mL of acetic anhydride was kept at room temperature for 48 h. After removal of solvents under vacuum without heat, the residue was chromatographed on silica gel with 3% diethyl ether in hexane, thereby yielding two main fractions.

3β-Acetoxy-10-hydroxy-6-oxo-5,6;5,10-disecocholestan-5-oic Acid Lactone (5→10). Rechromatography on μPorasil irrigated with hexane–isopropyl alcohol (24:1) at 1.0 mL/min of the first eluted fraction obtained from acetylation of 50 mg of **6a** (one-tenth of the fraction injected four times) gave 1.98 mg (37.6%) of **8** 3β-acetate as an oil: IR (CCl_4) 2730 (CHO), 1755 and 1730 (CO), 1245 cm^{-1}; ^1H NMR 0.70 (3 H, s, C-18), 1.39 (3 H, s, C-19), 2.08 (3 H, s, CH_3CO), 3.00 (1 H, 4-H), 5.14 (1 H, m, $W_{1/2}$ 10.3 Hz, 3α-H), 9.69 ppm (1 H, s, CHO); ^{13}C NMR 41.99 (C-4), 66.96 (C-3), 87.47 (C-10), 169.45 (C-5), 171.81 (CH_3CO), 203.59 ppm (C-6); EI mass spectrum, m/z (%) 476 (16.6) $(M)^+$, 458 (13.3) $(M - H_2O)^+$, 432 (14.6) $(M - CO_2)^+$, 416 (70) $(M - CH_3CO_2H)^+$, 398 (80) $(M - H_2O - CH_3CO_2H)^+$, 372 (16.6), 285 (23.3), 249 (20.6), 247 (18.7), 143 (100), 135 (33.3), 125 (13.3), 109 (14.6); R_f 0.27 (system IV), 0.56 (system III); t_R 15.3 min. Anal. Calcd for $C_{29}H_{48}O_5$: M, 476.35015. Found: M, 476.3498.

In like manner, there was obtained **8** 3β-acetate: 2.12 mg (41.5%) from **6b**, 1.83 mg (39.1%) from **6b** 3β-acetate, and 2.07 mg (45.4%) from **6c** 3β-acetate.

10-Hydroxy-6-oxo-5,6;5,10-disecocholest-3-en-5-oic Acid Lactone (5→10) (10). Following elution of **8** 3β-acetate from μPorasil, there was eluted 0.540 mg (11.7%) of **10** as an oil: UV (ethanol) 216 nm (ε 7200); IR (CCl_4) 2730 (CHO), 1730 and 1700 (CO), 1660 cm^{-1} (olefin); ^1H NMR 0.68 (3 H, s, C-18), 1.40 (3 H, s, C-19), 5.95 (1 H, d, $J = 12.8$ Hz, 4-H), 6.40 (1 H, dt, $J = 3.8$, 12.8 Hz, 3-H), 9.72 ppm (1 H, s, CHO); EI mass spectrum, m/z (%) 416 (7.3) $(M)^+$, 415 (4.2), 398 (14.6) $(M - H_2O)^+$, 388 (4.2), 382 (5.0), 372 (8.1), 332 (8.8), 318 (100), 303 (43.1), 285 (13.8), 249 (9.2), 247 (12.3), 149 (49.2), 135 (48.5), 125 (40.0); R_f 0.26 (system IV), 0.56 (system III); t_R 19.5 min. Anal. Calcd for $C_{27}H_{44}O_3$: M, 416.32903. Found: M, 416.3296.

In the same manner, 0.650 mg (14.6%) of **10** was recovered from 50 mg of **6b**, 0.44 mg (10.7%) of **10** from **6b** 3β-acetate, and 0.41 mg (10.3%) of **10** from **6c** 3β-acetate.

6,6-Diacetoxy-10-hydroxy-5,6;5,10-disecocholest-3-en-5-oic Acid Lactone (5→10) (11). The second main fraction eluted from silica gel after **8** 3β-acetate and **10** derived from **6a** gave 22.8 mg (39.8%) of **11** as an oil: UV (ethanol) 216 nm (ε 7800); IR (CCl_4) 1775, 1700, 1660, 1255 cm^{-1}; ^1H NMR 0.68 (3 H, s, C-18), 1.50 (3 H, s, C-19), 2.06 (6 H, s, CH_3CO), 5.98 (1 H, d, $J = 12.8$ Hz, 4-H), 6.42 (1 H, dt, $J = 3.8$, 12.8 Hz, 3-H), 6.96 ppm (1 H, t, $J = 5.1$ Hz, 6-H); ^{13}C NMR 86.40 (C-10), 90.22 (C-6), 123.78 (C-4), 144.35 (C-3), 165.99 (C-5), 168.91 and 169.09 ppm (CH_3CO); EI mass spectrum, m/z (%) 518 (0.28) $(M)^+$, 474 (0.23), 458 (30.6), 416 (53.3), 398 (100), 372 (25.3), 285 (22), 248 (25.3), 135 (58.7), 125 (72.7); R_f 0.17 (system IV), 0.42 (system III);

t_R 30.3 min. Anal. Calcd for $C_{31}H_{50}O_6$: C, 71.77; H, 9.71; M – CH_3CO_2H, 458.33959. Found: C, 71.84; H, 9.56; M – CH_3CO_2H, 458.3397.

3β,6ξ-Diacetoxy-10-hydroxy-6ξ-methoxy-5,6;5,10-disecocholestan-5-oic Acid Lactone (5→10) (12). The second main fraction eluted from silica gel after **8** 3β-acetate and **10** derived from **6b** gave 18.4 mg (31.2%) of **12** as an oil: IR (CCl_4) 1755, 1745, 1245 cm^{-1}; ^1H NMR 0.68 (3 H, s, C-18), 1.40 (3 H, s, C-19), 2.06 (6 H, s, CH_3CO), 3.00 (2 H, ABX, J = 1.7, 9.0, 14.1 Hz, 4-CH_2), 3.30 (3 H, s, OCH_3), 5.12 (1 H, m, $W_{1/2}$ 10.3 Hz, 3α-H), 5.87 ppm (1 H, dd, J = 4.6, 7.4 Hz, 6ξ-H); ^{13}C NMR 42.00 (C-4), 55.94 (OCH_3), 66.73 (C-3), 87.06 (C-10), 98.92 (C-6), 169.48 (C-5), 170.79 and 171.84 ppm (CH_3CO); EI mass spectrum, m/z (%) 518 (0.1) $(M - CH_3OH)^+$, 490 (3.0) $(M - CH_3CO_2H)^+$, 458 (1.5) $(M - CH_3OH - CH_3CO_2H)^+$, 430 $(M - 2CH_3CO_2H)^+$, 398 (4.7) $(M - CH_3OH - 2CH_3CO_2H)^+$, 263 (51.7), 249 (4.0), 248 (5.5), 247 (5.3), 143 (100), 135 (63.3), 125; R_f 0.13 (system IV), 0.41 (system III); t_R 25.5 min. Anal. Calcd for $C_{32}H_{54}O_7$: M – CH_3CO_2H, 490.365800. Found: M – CH_3CO_2H, 490.3652.[38]

In like manner, 50 mg of **6b** 3β-acetate yielded 16.2 mg (29.6%) of **12**.

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Registry No. 1a, 57-88-5; **1b**, 604-35-3; **3**, 81811-27-0; **5**, 20104-87-4; **5** 3,6-diacetate, 84680-99-9; **6a**, 81811-27-0; **6b**, 84681-00-5; **6b** 3-acetate, 84711-19-3; **6c**, 84681-01-6; **6c** 3-acetate, 84681-02-7; **6d**, 84681-03-8; **7a**, 1250-95-9; **7b**, 4025-59-6; **8**, 84681-04-9; **8** acetate, 84681-05-0; **10**, 84681-06-1; **11**, 84681-07-2; **12**, 84693-96-9.

Topological Charge Stabilization

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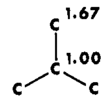
Abstract: The pattern of charge densities in a molecule is determined, at least in part, by the connectivity or the topology of the molecule. For the class of planar alternant hydrocarbons it is well-known that the calculated π-electron charge densities are all equal. But in nonalternant systems or in alternant systems for which the number of π electrons is not equal to the number of atomic orbitals that make up the system, the charge densities are not uniform. Many examples suggest that nature prefers to place atoms of greater electronegativity in those positions where the topology of the structure and the electron-filling level tend to pile up extra charge in the isoelectronic hydrocarbon. Since such heteroatomic systems are preferentially stabilized by molecular topology, the effect can be called the *rule of topological charge stabilization*. That such a rule indeed operates can be seen by comparing trends in calculated and empirical resonance energies, experimental heats of formation, and known relative molecular stabilities and reactivities to the patterns of charge densities calculated for the isoelectronic hydrocarbons. The topological charge stabilization rule has great potential value as a guide to synthetic efforts and as a quick way to rank the stabilities of positional isomers. It is easy to apply. Although more limited in its applicability than are energy quantities, the rule is often more direct because while energy is the property of a molecule as a whole, charge density is a property of an atom in a molecule. From a pattern of charge densities one can see immediately what is favorable or destabilizing about a particular arrangement of atoms. The simplicity and utility of this topological charge stabilization rule have gone largely unappreciated, although the idea was noticed at least as early as 1950 by Longuet-Higgins, Rector, and Platt.

The pattern of charge densities in a molecule is determined, at least in part, by the connectivity or the topology of the molecule. For the class of planar conjugated alternant hydrocarbons one can show quite generally that the simple Hückel π-electron charge densities are the same at all atoms in the molecule. But in nonalternant hydrocarbon systems or in alternant systems for which the number of π electrons is not equal to the number of atomic orbitals in the system, the charge densities are not all equal. These nonuniform charge densities arise solely from the way the atoms are connected and the number of electrons that fill the MO system. In this paper I will show many examples that suggest that nature prefers to place atoms of greater electronegativity in those positions where the topology of the structure tends to pile up extra charge. Since such heteroatomic systems are preferentially stabilized by molecular topology I call this the rule of *topological charge stabilization.*

Charge densities calculated for a heteroatomic molecule depend on the choice of semiempirical parameters, but in the isoelectronic hydrocarbon all Coulomb integrals are uniform and charge densities are determined only by topology and electron-filling level. To emphasize this fact I will refer to the isoelectronic hydrocarbon as the *uniform reference frame*, and all calculated charge densities reported here will be those calculated without assuming any heteroatomic parameters.[1]

Examples

Consider the case of the trimethylenemethyl dianion $C(CH_2)_3^{2-}$, in which a central carbon is connected to three peripheral carbons in planar geometry (**1**). The π-electron system is composed of



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six electrons moving through four atomic orbitals of the same type. The calculated Hückel π-electron charge densities are greater on the peripheral atoms (1.67) than on the central atom (1.00). The difference in charge densities can be easily understood from the nodal structure of the occupied molecular orbitals.[2] Recall that in simple Hückel theory charge density q_r at atom r is given by

$$q_r = \sum_i n_i c_{ir}^2 \qquad (1)$$

where c_{ir} is the coefficient of atomic orbital r in the molecular orbital i and n_i is the number of electrons in orbital i. Even for

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(1) Most Hückel charge densities mentioned here were taken from "Supplemental Tables of Molecular Orbital Calculations", A. Streitwieser, Jr., and J. I. Brauman, Eds., with a "Dictionary of π-Electron Calculations", C. A. Coulson and A. Streitwieser, Jr., Eds., Pergamon, Press, Oxford, 1965.

(2) B. M. Gimarc, "Molecular Structure and Bonding", Academic Press, New York, 1979, p 171.

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