presence of 0.127 g (0.137 mmol) of chlorotris(triphenylphosphine)rhodium in a sealed tube at 60 °C. The predescribed workup, followed by purification by liquid chromatography on silica gel with 7:3 petroleum ether/benzene as an eluent, gave 1.02 g (62%) of 10-acetyllimonene[9] (8): IR (neat) 1715 cm^{-1} (CO); MS, m/z (relative intensity) 178 (M^+, 2), 43 (100); ^1H NMR ($CDCl_3$) δ 1.64 (3 H, br s), 2.10 (3 H, s), 3.16 (2 H, s), 4.88 and 5.00 (2 H, m), 5.37 (1 H, m).

With Senecioyl Chloride. A solution of 2 g (6.7 mmol) of 10-(trimethylstannyl)limonene (2) and 1.19 g (10 mmol) of senecioyl chloride in 4 mL of dichloromethane are heated (60 °C) for 16 h in a sealed tube in the presence of 0.062 g (0.067 mmol) of rhodium complex. The reaction mixture was worked up as in the experiments above. The crude product obtained after removing the solvent was purified by liquid chromatography on silica gel.

Elution with 6:4 petroleum ether/benzene gave 680 mg (47%) of β-atlantone (9):[12,13] IR (neat) 1685 cm^{-1} (CO); MS, m/z (relative intensity) 218 (M^+, 3), 83 (100); ^1H NMR ($CDCl_3$) δ 1.62 (3 H, br s), 1.81 (3 H, br s), 2.10 (3 H, br s), 3.10 (2 H, br s), 4.80 and 4.90 (2 H, m), 5.30 (1 H, m), 6.00 (1 H, m).

Further elution with 4:6 petroleum ether/benzene gave 220 mg (15%) of α-atlantone (10):[12,13] IR (neat) 1685 cm^{-1} (CO); MS, m/z (relative intensity) 218 (M^+, 14), 83 (100); ^1H NMR ($CDCl_3$) δ 1.62 (3 H, br s), 1.85 (3 H, br s), 2.13 (6 H, br s), 5.36 (1 H, m), 5.97 (2 H, m).

Acylation of 10-(Trimethylstannyl)-2-carene (3) with Senecioyl Chloride. A solution of 1.5 g (5 mmol) of 10-(trimethylstannyl)-2-carene (3) and 0.89 g (7.5 mmol) of senecioyl chloride in 3 mL of dichloromethane was allowed to react in the presence of 0.047 g (0.05 mmol) of rhodium complex in a sealed tube at 60 °C for 24 h.

The reaction mixture was worked up as previously described and was purified by liquid chromatography on silica gel with 2:3 petroleum ether/benzene as an eluent to give 560 mg (51%) of 2-α-senecioyl-3(10)-carene (11): IR (neat) 1685 cm^{-1} (CO); MS, m/z (relative intensity) 218 (M^+, 3), 83 (100); ^1H NMR ($CDCl_3$) δ 0.95 (3 H, s), 1.02 (3 H, s), 1.88 (3 H, br s), 2.11 (3 H, br s), 3.44 (1 H, br s), 4.86 (2 H, m), 6.22 (1 H, m). Anal. Calcd for $C_{15}H_{22}O$: C, 82.57; H, 10.10. Found: C, 82.47; H, 10.19.

Acylation of 7-(Trimethylstannyl)-p-cymene (4). The reactions were conducted in the same fashion as with the allylstannanes. A solution of 0.0106 mol of acid chloride (acetyl or senecioyl chloride) and 0.0067 mol of 7-(trimethylstannyl)-p-cymene (4) in 5 mL of dichloromethane in the presence of 0.067 mmol of chlorotris(triphenylphosphine)rhodium is heated (60 °C) in a sealed tube for 48 h. The solution was cooled to room temperature, poured into a saturated aqueous NH_4Cl solution, and extracted with ether. The organic layer was washed with H_2O and dried ($MgSO_4$). The solvents were removed by evaporation and the crude product was purified by liquid chromatography on silica gel with 4:6 petroleum ether/benzene as an eluent.

With Acetyl Chloride: 850 mg (72%) of 7-acetyl-p-cymene (12) was obtained; MS, m/z (relative intensity) 176 (M^+, 21), 133 (100); ^1H NMR ($CDCl_3$) δ 1.18 (6 H, d), 2.05 (3 H, s), 3.55 (2 H, s), 7.05 (4 H, s). Anal. Calcd for $C_{12}H_{16}O$: C, 81.82; H, 9.09. Found: C, 82.03; H, 9.11.

With Senecioyl Chloride: 500 mg (53%) of 7-senecioyl-p-cymene (13) was obtained; MS, m/z (relative intensity) 216 (M^+, 3), 83 (100); ^1H NMR ($CDCl_3$) δ 1.24 (6 H, d), 1.40 (3 H, br s), 2.15 (3 H, br s), 2.82 (1 H, m), 3.66 (2 H, s), 6.08 (1 H, m), 7.13 (4 H, s). Anal. Calcd for $C_{15}H_{20}O$: C, 83.33; H, 9.26. Found: C, 83.51; H, 9.15.

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Registry No. 1, 105760-49-4; 2, 112069-27-9; 3, 100692-36-2; 4, 105631-51-4; 5, 64615-27-6; 8, 112069-28-0; 9, 38331-79-2; 10, 112137-48-1; 11, 112069-29-1; 12, 7306-39-0; 13, 112069-30-4; α-pinene, 80-56-8; limonene, 5989-27-5; 2-carene, 554-61-0; p-cymene, 99-87-6.

Ozonization of Cholesterol in Nonparticipating Solvents[1]

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Reaction of ozone with cholesterol (or cholesterol 3β-acetate) in dilute CCl_4 or hexane solutions gave the heretofore undescribed isomeric 1,2,4-trioxolane ozonides 5,7α-epidioxy-5α-*B*-homo-6-oxacholestan-3β-ol and 5,7β-epidioxy-5β-*B*-homo-6-oxacholestan-3β-ol (or their 3β-acetates). Structures are supported by proton and carbon spectra and by Zn/acetic acid reduction to 3β-hydroxy(or 3β-acetoxy)-5-oxo-5,6-secocholestan-6-al. At higher cholesterol concentrations oxidized dimeric and oligomeric products are formed at the expense of 1,2,4-trioxolane ozonides. The major dimer 6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol formed monoesters and was reduced by Zn/acetic acid to equivalent amounts of 3β-hydroxy-5-oxo-5,6-secocholestan-6-al and cholesterol. Also formed from cholesterol were dimeric ozonides 6ξ-(5′,7′α-epidioxy-5′α-*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol and 6ξ-(5′,7′β-epidioxy-5′β-*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol and dimeric epoxides 5,6ξ-epidioxy-6ξ-(5′,6′α-epoxy-5′α-cholestan-3′β-yloxy)-5ξ-5,6-secocholestane-3β,5-diol and 5,6ξ-epidioxy-6ξ-(5′,6′β-epoxy-5′β-cholestan-3′β-yloxy)-5ξ-5,6-secocholestane-3β,5-diol. Oligomeric 1,2,4-trioxolane ozonide isomers were also formed. Ozone thus reacts with cholesterol in two ways, by epoxidation and putatively by carbonyl oxide formation, with subsequent carbonyl oxide cyclization to 1,2,4-trioxolane ozonides or interception of cholesterol, cholesterol epoxides, and cholesterol ozonides (as alcohols) to yield dimeric and oligomeric species.

The reaction of ozone (O_3) with cholesterol (1a) (Chart I) and with cholesterol 3β-acetate (1b) in nonparticipating solvents for form 5,6-secosterols has received attention from 1905, but only poorly characterized putative ozonides,

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overozonized products, or further transformed derivatives have been described.[3] More recent examinations of the reaction with cholesterol established that peroxidic products were formed but that the desired 1,2,4-trioxolane ozonides 5,7α-epidioxy-5α-*B*-homo-6-oxacholestan-3β-ol (**2**) and 5,7β-epidioxy-5β-*B*-homo-6-oxacholestan-3β-ol (**3**) were not.[4,5] In the present paper we establish that cholesterol does indeed form the isomeric 1,2,4-trioxolane ozonides **2** and **3** in suitably diluted nonparticipating solvents but that dimeric and oligomeric oxidation products also form.

Results

In CCl_4 or hexane the reaction between O_3 and cholesterol was complete within 30 min at room temperature, with formation of at least ten difficultly separated peroxidic components chromatographically more mobile than cholesterol. Oxidation products more polar than cholesterol were present only at very low levels, and none was recognized as cholesterol ozonide or as a common cholesterol autoxidation product.[6]

As oxidized secosterols less mobile than cholesterol were expected, the more mobile products obtained from CCl_4 solutions were initially viewed as possibly arising through incorporation of solvent or perhaps by chlorination.[7] However, the products were devoid of halogen (Beilstein test), and the observed chromatographic properties were suitably rationalized upon recognition of their dimeric and oligomeric natures. The products were stable peroxides with constant chromatographic and spectral properties, thus not like oligomers without specific structures described in some olefin ozonizations.[8]

The initially formed, major product for which the dimer structure 6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (**4a**) is proposed readily formed monoesters 3β-acetoxy-6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (**4b**) and 3β-[(*p*-bromobenzoyl)oxy]-6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (**4c**). The monoacetate **4b** was also isolated from the acetylated total product mixture. Reduction of **4a** (or **4b**) by $(CH_3)_2S$ or Zn/acetic acid gave equal amounts of cholesterol and 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (**5**) (or **5** 3β-acetate).

Proton and carbon spectra established the dimeric nature of **4**. Two C-18 and two C-19 angular methyl groups are evinced, one C-19 methyl having the same chemical shifts as that of cholesterol, the other with a proton signal at δ 1.0 and a carbon signal at δ 16.0 (Table I), thus deshielded and shielded, respectively, from their positions in spectra of cholesterol.[9]

Additionally, six unique one-proton signals were prominent in the spectra of **4**. A doublet (J = 5.5 Hz) at δ 5.3 in the spectra of **4** and a broad multiplet ($W_{1/2}$ = 24 Hz) at δ 3.84 in the spectrum of **4a** shifted to δ 4.86 in the spectrum of **4b** evinced vinyl proton and acylable secondary alcohol features, respectively, thus retention of Δ^5-double bond and 3β-alcohol group in **4a**. Carbon signals at δ 140 and 122 in the spectra of **4** and at δ 66.7 in the spectrum of **4a** shifted to δ 69.7 in that of **4b** confirmed, respectively, the presence of the Δ^5-double bond and acylable 3β-alcohol features.[10] Sharp infrared absorption at 3640 cm^{-1} (O–H stretching) in CCl_4 was not altered on dilution and a broad band at 3425 cm^{-1} disappeared on dilution (intermolecular hydrogen-bonded hydroxyl), further confirming the 3β-hydroxyl feature of **4a**.[11]

A second broad multiplet at δ 3.63 in the proton spectrum of **4a** unshifted in the spectrum of **4b** was recognized as arising from a second 3α-hydrogen of sterol 3β-alcohol present as an ether. A carbon signal attributable to the putative 3β-ether function was not directly observed, as the signal exactly overlay the δ 77.43 signal of solvent deuteriochloroform. The δ 77.43 signal was more intense than the others (at δ 77.00 and 76.57) of the triplet (approximately equal intensities are expected). Moreover, the attached proton test (APT) pulse sequence[12] in which methine carbon signals appear in the spectra directed downward completely nulled the δ 77.43 solvent signal, confirming that a signal from the carbon bearing oxygen fortuitously resonated exactly at the same frequency as the solvent.

The three remaining one-proton signals were recognized as associated with the oxygen features of an oxidized 5,6-secosterol. A broad doublet or doublet of doublets at δ 2.58–2.75 in the spectra of **4** was identified as representing the hydrogen vicinal to the carbon bearing newly introduced oxygen, specifically the 4α-hydrogen.[13]

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(2) Robert A. Welch Foundation Post-Doctoral Fellow 1983–1986 on leave from the Department of Fundamental Chemistry, Academy of Agriculture, Wroclaw, Poland.

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(6) Free radical autoxidation did not accompany the O_3 reaction in either protic participating or nonparticipating solvent systems, as neither cholesterol 7-hydroperoxides, cholest-5-ene-3β,7-diols, nor dimeric species bearing these features were detected among reaction products.

(7) Resinous products containing 5.5–6.5% Cl were obtained by Lettré and Jahn, cf. ref 4, in ozonization of **1b** in $CHCl_3$ or CCl_4.

(8) Greenwood, F. L.; Rubinstein, H. *J. Org. Chem.* **1967**, *32*, 3369–3374.

(9) Proton spectra of diverse symmetrical sterol dimers exhibit only one C-18 and one C-19 signal, one vinyl proton signal, one signal for hydrogen geminal to hydroxyl or ether oxygen, etc.; cf. (a) Pinkus, J. L.; Cohen, T. *J. Org. Chem.* **1968**, *33*, 3538–3541. (b) Stohrer, G.; *Steroids* **1971**, *17*, 587–594. (c) Boomsma, F.; Jacobs, H. J. C.; Havinga, E.; Van Der Gen, A. *Recl. Trav. Chim.* **1973**, *92*, 1361–1367. (d) Tal, D. M.; Keinan, E.; Mazur, Y. *Tetrahedron* **1981**, *24*, 4327–4330. However, unsymmetric sterol dimers give rise to doubled signals for these features; cf. ref 8c and (e) Blunt, J. W.; Hartshorn, M. P.; Kirk, D. N. *Tetrahedron* **1966**, *22*, 3195–3202. (f) Crabbé, P.; Mislow, K. *J. Chem. Soc. D* **1968**, 657–658. Compounds such as 3α-(cholest-5′-en-3′β-yloxy)-*A*-homo-4-oxacholest-5-ene containing a cholesterol moiety bonded to an oxidized sterol moiety exhibit proton signals of each part independently; cf. (g) Suginome, H.; Furusaki, A.; Kato, K.; Maeda, N.; Yonebayashi, F. *J. Chem. Soc., Perkin Trans. 1* **1981**, 236–250.

(10) Acetylation of a 3β-hydroxysterol causes a deshielding of the C-3 carbon by ca. 2.3 ppm. Adjacent C-2 and C-4 carbons are shielded by ca. 4 ppm; cf. Blunt, J. W.; Stothers, J. B. *Org. Magn. Reson.* **1977**, *9*, 439–464.

(11) Under identical conditions spectra of cholesterol and **6** in CCl_4 had sharp bands at 3640 cm^{-1} not affected by dilution and broad bands at 3400–3450 cm^{-1} disappearing on dilution. Neither band was present in the spectra of **4b**, **1b**, or **6** 3β-acetates.

(12) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535–539.

(13) Similar doublet or doublet of doublets signals assigned to the 4α-hydrogen resonance occur in spectra of (a) 3β-acetoxy-5,8-epoxy-5α,8α-7-oxacholestan-6α-ol at δ 2.44; cf. Gumulka, J.; Szczepek, W. J.; Wielogorski, Z. *Tetrahedron Lett.* **1979**, *50*, 4847–4850; *Polish J. Chem.* **1983**, *57*, 403–411. (b) 6ξ-Alkoxy-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diols **6** and their 3β-acetates at δ 2.62–2.68; cf. ref 5.

We have confirmed assignment of the doublet of doublets at δ 2.6 in the spectrum of **6a** 3β-acetate to the 4α-hydrogen, strongly coupled to the 4β-hydrogen (δ ca. 1.65), more weakly to the 3α-hydrogen, by means of the homonuclear correlation spectroscopy (COSY) pulse sequence of Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246.

Table I. Carbon Spectral Data of Cholesterol Derivatives[a,b]

							4a		4b				8		9	
C	1a	1b	2	2 3β-Ac	3	3 3β-Ac	seco moiety	Δ⁵ moiety	seco moiety	Δ⁵ moiety	6a	6a 3β-Ac	seco moiety	6-oxa moiety	seco moiety	6-oxa moiety
1	37.14	36.85	33.01	32.65	32.97	34.84	33.58	37.20	33.19	37.20	33.60	33.22	33.58	32.91	33.60	33.60
2	31.47	27.64	29.83	26.05	30.35	26.97	30.71	29.39	27.00	29.34	30.65	27.00	30.72	27.97	30.73	28.62
3	71.52	73.79	68.69	70.88	68.34	70.48	66.82	77.43	69.70	77.36	66.83	69.70	66.83	75.14	66.84	74.17
4	42.12	37.97	41.12	37.22	39.44	35.64	39.63	36.13	35.73	36.10	39.23	35.50	39.45	35.74	39.46	35.72
5	140.62	139.44	112.33	111.80	113.11	112.23	111.78	140.11	111.42	140.13	111.89	111.56	111.85	112.05	111.84	112.49
6	121.44	122.48	101.09	101.06	103.00	103.07	98.62	122.15	98.55	122.02	102.38	102.39	99.62	101.18	99.07	103.11
7	31.75	31.73	39.37	39.41	39.44	39.24	38.58	31.88	38.43	31.87	39.84	39.77	38.06	39.32	39.46	39.75
8	31.75	31.73	34.34	34.35	35.70	35.55	33.64	31.81	33.62	31.78	33.49	33.49	33.58	34.34	33.60	34.86
9	50.01	49.89	46.54	46.47	48.73	48.68	47.73	50.08	47.68	50.10	47.75	47.68	47.75	46.51	47.75	48.72
10	36.33	36.42	42.46	42.32	43.01	42.55	44.36	36.68	44.42	36.65	44.40	44.47	44.38	42.37	44.37	42.63
11	20.96	20.90	22.34	22.25	23.69	22.45	22.55	20.99	22.45	20.96	22.54	22.52	22.53	22.28	22.53	22.53
12	39.64	39.59	41.12	41.75	39.56	39.41	40.74	39.69	40.65	39.67	39.45	39.40	40.63	42.14	40.57	42.46
13	42.12	42.15	41.82	42.09	42.46	42.44	41.75	42.28	41.72	42.23	41.73	41.72	41.75	41.75	41.73	41.73
14	56.63	56.54	55.51	55.39	55.98	55.97	54.89	56.67	54.82	56.66	54.88	54.82	54.86	55.44	54.84	55.98
15	24.15	24.12	26.10	26.05	25.92	25.88	26.10	24.23	26.05	24.18	26.04	26.04	26.08	26.08	26.08	25.91
16	28.10	28.10	27.70	27.64	27.72	27.67	27.55	28.17	27.52	28.13	27.58	27.57	27.58	27.70	27.59	27.72
17	56.05	56.00	56.00	55.94	56.19	57.61	56.11	56.11	56.08	56.08	56.10	56.08	56.14	55.98	56.11	57.63
18	11.71	11.72	11.47	11.43	11.49	11.46	11.47	11.78	11.46	11.77	11.48	11.47	11.46	11.46	11.48	11.48
19	19.26	19.14	18.64	18.57	18.53	18.00	16.07	19.28	15.98	19.26	16.04	15.97	16.07	18.59	16.10	17.20
20	35.64	35.64	35.74	35.67	35.70	35.64	35.72	35.72	35.67	35.67	35.64	35.64	35.74	35.74	35.72	35.78
21	18.60	18.57	18.64	18.57	18.63	18.60	18.57	18.63	18.54	18.63	18.58	18.57	18.59	18.59	18.61	18.61
22	36.07	36.04	36.03	35.96	35.90	35.99	35.98	35.98	35.96	35.96	35.95	35.95	35.95	35.98	35.99	35.99
23	23.72	23.69	23.76	23.72	23.76	23.72	23.78	23.78	23.75	23.75	23.73	23.77	23.77	23.77	23.76	23.76
24	39.38	39.38	39.49	39.27	39.44	39.47	39.46	39.46	39.44	39.44	39.45	39.45	39.45	39.45	39.46	39.46
25	27.84	27.84	27.98	27.90	27.97	27.89	27.95	27.95	27.93	27.93	27.97	27.97	27.97	27.97	27.98	27.98
26	22.68	22.68	22.80	22.71	22.78	22.71	22.77	22.77	22.71	22.71	22.79	22.78	22.79	22.79	22.80	22.80
27	22.43	22.43	22.53	22.45	22.45	22.45	22.45	22.45	22.45	22.45	22.54	22.52	22.53	22.53	22.53	22.53
CO		170.24		170.04		170.13			170.07			170.24				
CH_3		21.27		21.16		21.19			12.27			21.33				
OCH_3											55.70	55.73				

[a] Original carbon numbers of cholesterol. Carbons C6 and C7 listed for the 6-oxa-*B*-homo sterols **2**, **3** and their 3β-actates and the 6-oxa-*B*-homo moiety of **8** and **9** are formally C7 and C7a. [b] Seco moiety refers to the 5,6-secosterol unit bearing the 5,6-epidioxide feature analogous to that in **6**.

A doublet of doublets at δ 4.86–4.91 in the spectra of **4** similar to signals in the spectra of sterol and triterpenoid 1,2,4-trioxolane ozonides[14] evinced the presence of hydrogen attached to carbon doubly substituted by oxygen, the feature being confirmed by carbon spectra. The sixth unique one-proton signal was a sharp singlet at δ 10.3 not shifted by dilutions but disappearing upon treatment with ^2H_2O, correlated with a 3320 cm^{-1} absorption also unaffected by dilutions. One other doubly oxygenated carbon atom was indicated by carbon spectra, it being obvious that the two doubly oxygenated carbons were the C-5 and C-6 atoms of the original Δ^5-double bond of cholesterol.

These spectral features of **4** resemble in detail those of 6ξ-alkoxy-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diols (**6**) and their 3β-acetates obtained by ozonization of cholesterol and **1b** in participating hydroxylic solvents.[4,5,15] The prominent absorption bands in the spectrum of **4a** at 3540 and 3425 cm^{-1} (absent in the spectra of **4b** and **4c**) previously mentioned are exactly analogous to the 3540 and 3400–3450 cm^{-1} bands in the spectra of cholesterol and secosterols **6** (absent from spectra of **1b** and **6** 3β-acetates) and establish the 3β-hydroxyl feature of **4a**. The additional prominent band at 3320 cm^{-1} unaltered by dilution in the spectra of **4** is also present in the spectra of **6** and **6** 3β-acetates.[15]

Moreover, proton spectra of **4**, **6**, and **6** 3β-acetates are very much alike, only the multiplicity of the δ 4.6–5.0 signal varying.[16] The sharp one-proton signals near δ 10.3 in the spectra of **4** are exactly like those of the δ 10.0–10.8 signals in spectra of **6** and **6** 3β-acetates.[15] Additionally, carbon spectra of **4** correspond with those of **6** and **6** 3β-acetates (Table I); the only prominent difference is the C-6 signal of **4** appreciably more shielded than those of 5,6ξ-epidioxy-6ξ-methoxy-5ξ-5,6-secocholestane-3β,5-diol (**6a**) and 5,6ξ-epidioxy-6ξ-ethoxy-5ξ-5,6-secocholestane-3β,5-diol (**6b**) and their 3β-acetates but deshielded in comparison with 6ξ-*tert*-butoxy-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (**6d**).

These spectral details support the assigned 6ξ-alkoxy-5,6-epidioxy-5ξ-alcohol structure of **4** as do also mass spectra. However, mass spectra of **4** are dominated by ions readily identified as those of cholesterol (or **1b**)[17] and differ in some details from spectra of **6** and **6** 3β-acetates. The electron impact (EI) mass spectrum of **4a** includes elimination ions m/z 402 $(M - ROH - O_2)^+$ and 386 $(ROH)^+$ but not the $(M - H_2O)^+$, $(M - ROH)^+$, and $(M - H_2O - ROH)^+$ ions in the spectra of **6**. The chemical ionization (CI) mass spectrum of **4a** reveals ions m/z 417 $(M - H_2O - ROH + H)^+$ and 399 $(M - 2H_2O - ROH + H)^+$ present in CI spectra of **6**.[5,15]

Moreover, elimination ions m/z 416 $(M - ROH - CH_3CO_2H)^+$ and 386 $(ROH)^+$ were found in the EI mass spectrum of **4b** but not the $(M - H_2O)^+$, $(M - ROH)^+$, and $(M - H_2O - ROH)^+$ ions in the EI spectra of **6** 3β-acetates. The CI mass spectrum of **4b** reveals ions m/z 459 $(M - H_2O - ROH + H)^+$ and 399 $(M - H_2O - ROH - CH_3CO_2H + H)^+$ common to CI spectra of **6** 3β-acetates.[5] Clearly, dimers **4** are more sensitive to bond cleavages under both EI and CI conditions than are **6** and **6** 3β-acetates.

Ozonization of the 3β-acetate **1b** took another course, with no dimeric materials formed. Rather, two more polar peroxidic products resolved from one another only with repeated recycling HPLC were identified as the long sought but undescribed isomeric 1,2,4-trioxolane ozonides **2** 3β-acetate and **3** 3β-acetate, obtained in 2:1 ratio. These results establish that ozonization of **1b** in nonparticipating solvents yields the predicted isomeric 1,2,4-trioxolane ozonides and suggest that formation of dimer **4a** but not ozonides **2** and **3** in the ozonization of cholesterol result from reaction of unoxidized cholesterol as an alcohol with a reactive oxidized sterol intermediate. Reexamination of the ozonization of cholesterol in dilute CCl_4 or hexane solutions confirmed this viewpoint, with recovery of the required 1,2,4-trioxolane ozonides **2** and **3**, also in 2:1 ratio. By increasing the concentration of cholesterol in the reacting system both ozonides **2** and **3** and dimer **4a** were formed, the amounts of **2** and **3** declining with increasing concentration of cholesterol until dimer **4a** became the predominant reaction product.

Ozonides **2** and **3** and their 3β-acetates were stable for simple manipulations and HPLC but decomposed to unidentified products in a matter of days to weeks at room temperature whether in solution or as solids. By contrast ozonide derivatives **4a**, **6**, and their 3β-acetates are stable for years as solids.[18]

Structures of ozonides **2** and **3** and their 3β-acetates were established by Zn/acetic acid reductions of each to **5** or **5** 3β-acetate and by infrared, proton, and carbon spectra (Table I). Infrared spectra evinced the presence of the 3β-hydroxyl group in **2** and **3** and of the 3β-ester in **2** and **3** 3β-acetates, confirmed by proton and carbon spectra. Additionally, the absence of olefin features but the presence of two carbon atoms doubly substituted by oxygen, only one of which having a single attached hydrogen, support the structure assignments.

The ozonides **2** and **3** from cholesterol were readily correlated with the corresponding ozonide 3β-acetates from **1b** by proton and carbon spectra, and acetylation of the less mobile ozonide **3** gave a monoacetate (**3** 3β-acetate) identical with the more mobile of the ozonide 3β-acetates from **1b**, thus confirming the formulation. Acetylation of the more mobile ozonide **2** did not yield a monoacetate, but other products as yet unidentified were formed.

Stereochemistry of ozonides **2** and **3** and their 3β-acetates was assigned tentatively from three elements of proton spectra. The broad multiplet character of the 3α-proton signal in all spectra suggests an axial 3α-hydrogen in A-ring chair conformation, and Dreiding molecular models readily accommodate these features in all cases. In either isomer model the 7ξ- and 7aβ-hydrogens are equatorial, being eclipsed in the 5α,7α-ozonide model (bearing the 5α,7α-epidioxide feature), thus giving a larger 7β-proton coupling constant than the isomeric 5β,7β-ozonide model (bearing the 5β,7β-epidioxide feature) where the 7α-H/C-7/C-7a/7aβ-H dihedral angle is about 30°. By this consideration, the more mobile ozonide is the 5α,7α-ozonide **2**, the less mobile isomer the 5β,7β-ozonide **3**.

Supporting this assignment is the distinctive deshielded doublet of doublets doubled at δ 2.2 of the 7aα-proton in

(14) (a) Wife, R. L.; Kyle, D.; Mulheirn, L. J.; Volger, H. C. *J. Chem. Soc., Chem. Commun.* **1982**, 306–307. (b) Miljković, D.; Čanadi, D.; Petrović, J.; Stanković, S.; Ribar, B.; Argay, G.; Kálmán, A. *Tetrahedron Lett.* **1984**, *25*, 1403–1406. (c) Ageta, H.; Shiojima, K.; Kamaya, R.; Masuda, K. *Tetrahedron Lett.* **1978**, 899–900.

(15) Jaworski, K.; Smith, L. L. *Magn. Reson. Chem.*, in press.

(16) The signal is a doublet of doublets in spectra of **4**, **6c**, **6d**, and **6d** 3β-acetate but a triplet in spectra of **6a**, **6b**, and their 3β-acetates. However, the signal is a doublet of doublets (δ 4.71, J = 6.0, 9.9 Hz) in spectra of **6a** and **6a** 3β-acetate in deuterioacetone.

(17) Mass spectra of 3β-alkoxyalkoxy ethers of cholesterol do not reveal molecular ions but only ions of cholesterol and lower mass ions, cf. Herz, J. E.; Lucero, J.; Santoyo, Y.; Waight, E. S. *Can. J. Chem.* **1971**, *49*, 2418–2419.

(18) Samples of **6** and **6** 3β-acetates stored at room temperature since 1983 remain unaltered. Solutions of **4** or **6** in deuteriochloroform for several weeks at room temperature eventually decompose, as indicated by growth of a proton singlet at δ 9.6 attributed to the 6-aldehyde proton of **5** or **5** 3β-acetate.

the spectra of the more mobile ozonide and its 3β-acetate evincing coupling to three protons, the 7β-, 7aβ-, and 8β-hydrogens.[19] The 7β-H/C-7/C-7a/7aα-H dihedral angle of ca. 120°, 7a-protons geminal nature, and trans-diaxial configuration of the 7aα- and 8β-hydrogens, respectively, allow such coupling. By contrast, the corresponding doublet or doublet of doublets of the 7aα-proton near δ 2.1 in the spectra of the less mobile ozonide and its 3β-acetate disclose coupling to but two protons. From the β-ozonide model the 7α-H/C-7/C-7a/7aα-H dihedral angle of 90° presumably removes 7α- and 7aα-proton coupling from spectra, thus confirming the β-ozonide structure **3** for the less mobile ozonide.

From these assignments other distinctive spectral features may be correlated with stereochemistry. Thus, the 5β,7β-epidioxide feature of **3** shields the C-19 protons relative to the isomeric 5α,7α-epidioxide feature of **2**. Furthermore, the C-4 of **3** is deshielded and the C-7, C-7a, C-8, and C-9 of **3** are shielded relative to the corresponding carbons of **2**.

It is thus apparent that cholesterol participates in the ozonization in nonparticipating solvents in two separate ways, as an oxidizable olefin and as an alcohol reacting with a reactive, oxidized secosterol intermediate, presumably the Criegee carbonyl oxide 3β-hydroxy-5-oxo-5,6-secocholestane-6-carbonyl oxide (**7**).[20] Intramolecular interception of the 6-carbonyl oxide feature by the 5-ketone group of **7** is inferred by formation of the isomeric 1,2,4-trioxolane ozonides **2** and **3** in dilute solutions (less than 2 mM). However, formation of dimer **4a** from cholesterol additionally infers intermolecular interception of the 6-carbonyl oxide moiety of **7** (or other reactive oxidized secosterol) by the 3β-hydroxyl group of cholesterol, interception by hydroxyl predominating at higher cholesterol concentrations (50 mM). Both processes compete at intermediate concentrations, yielding ozonides **2** and **3** and dimer **4a**. Neither ketone nor 3β-hydroxyl group competed in interception of the carbonyl oxide with small amounts of methanol added to the ozonization, **6a** being formed exclusively in the presence of methanol.[21]

Alternatively, reaction of cholesterol with ozonides **2** or **3** to yield dimer **4a** might occur in analogy to the reaction of other 1,2,4-trioxolanes with methanol,[22] yielding dimer 6ξ-(cholest-5′-en-3′β-yloxy)-3β-hydroxy-5ξ-*B*-homo-6-oxacholestane 5-hydroperoxide or the regioisomeric 5ξ-(cholest-5′-en-3′β-yloxy) 6ξ-hydroperoxide. However, ozonides **2** and **3** in CCl_4 or hexane solutions containing up to a tenfold excess of cholesterol were not altered overnight at room temperature; neither was **2** or **3** in methanol solution altered by like treatment. We conclude that this reaction mode does not account for the formation of dimer **4a** during ozonization nor does a putative hydroperoxide product form.[23] Thus, dimer **4** results from reaction of carbonyl oxide **7** with cholesterol acting as an alcohol in the same manner in which 6-alkoxy-5,6-epidioxy-5,6-secosterols **6** (now recognized as lower homologues of **4a**) form.

The C-5 and C-6 stereochemistry of the 6ξ-(cholesteryloxy)-5ξ,6ξ-epidioxy 3β,5ξ-diol structure of **4a** cannot now be assigned from spectra, and Dreiding molecular models in either C-5 configuration suggest conformational mobility of the eight-membered B-ring of **4** and **6**. The doublet of doublets multiplicity of the 6ξ-hydrogen signal of **4**, **6c**, and **6d** compared with the triplet signal of **6a** and **6b** could reflect different C-6 stereochemistry but may merely represent steric influences on the B-ring conformation of the more bulky 6ξ-alkoxyl groups of **4**, **6c**, and **6d**. However, different C-5 stereochemistry may occur among **4** and **6**, as the behavior of **4a** to acylation conditions is different from that of **6a** and **6b**, **4a** yielding monoesters **4b** and **4c** smoothly, **6a** and **6b** rearranging to *A*-ring bissecolactones.[5]

In either C-5 configuration the close proximity of the 5ξ-hydroxyl proton of **4** and **6** to the more remote oxygen of the vicinal 5,6-epidioxide or the 6ξ-alkoxyl oxygen, in a 1,5- or 1,7-hydrogen bond, respectively, is evinced by 3280–3330 cm^{-1} absorption, the intramolecular association holding the 5ξ-hydroxyl proton within the deshielding region of the epidioxide oxygen atoms thus accounting for the low field δ 10.0–10.8 sharp singlets observed. As only one such proton singlet and associated hydroxyl absorption band are observed, each secosterol **4** and **6** must exist exclusively in but one conformation in a relatively stable state.[18,24]

Several minor products are also obtained from the ozonization of cholesterol, some of which are formed after **4a** and appear to derive from **4a**. Two of these were also major products of the ozonization of **4a** in an experiment conducted separately to test this point and were recognized as isomeric 1,2,4-trioxolane ozonides of **4a**, thus 6ξ-(5′,7′α-epidioxy-5′α-*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (**8**) and isomeric 6ξ-(5′,7′β-epidioxy-5′β-*B*′-homo-6′-oxacholestan-3′β-

(19) Homonuclear correlated spectroscopy (COSY) data show the 7β-proton is coupled to the proton giving rise to the δ 2.2 doublet of doublets doubled, thus identifying the 7aα-proton.

(20) It is assumed that cholesterol ozonization involves formation of the Criegee carbonyl oxide; cf. (a) Murray, R. W. *Acc. Chem. Res.* **1968**, *1*, 313–320. (b) Criegee, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 745–752. (c) Bailey, P. S. *Ozonization in Organic Chemistry*; Academic Press: New York, 1978, Vol. I, pp 15–30; 1982, Vol. II, pp 371–424. The structure of the carbonyl oxide formed in participating alcoholic solvents as **7** but not as the regioisomeric 6-oxo-5-carbonyl oxide is supported by recovery of 3β-acetoxy-6ξ-ethoxy-6ξ-hydroxy-5,6-secocholestan-5-one from catalytic reduction of 3β-acetoxy-5,6ξ-epidioxy-6ξ-ethoxy-5ξ-5,6-secocholestan-5-ol [cf. (d) Lettré, H.; Jahn, A.; Pfirrmann, R. *Liebigs Ann. Chem.* **1958**, *615*, 222–227] and by derivation of 3β,6ξ-diacetoxy-10-hydroxy-6ξ-methoxy-5,6;5,10-bissecocholestan-5-oic acid lactone (5→10) upon treatment of **6a** with acetic anhydride/pyridine, cf. ref 5.

The putative regioisomeric 6-oxo-5-carbonyl oxide alternative is also eliminated as precursor of **4** in nonparticipating solvents by carbon spectra, the C-6 signal of **4** but not the C-5 signal being shielded from the corresponding signal in spectra of **6a** and **6a** 3β-acetate.

(21) Intermolecular interception of the carbonyl oxide by cholesterol should be more suggish than interception by lower primary alcohols cf. Yamamoto, Y.; Niki, E.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2677–2678. Other carbonyl oxide interceptions would yield unlikely products or products not supported by the spectral evidence; thus, intramolecular interception of the 6-carbonyl oxide of **7** by the 3β-hydroxyl although allowed by assembly of Dreiding molecular models results in a highly strained system and is accordingly excluded. Intermolecular interception by the 5-ketone or self-interception of the carbonyl oxide would yield dimeric or oligomeric peroxyketones not supported by spectral evidence.

(22) (a) Foote, C. S.; Wuesthoff, M. T.; Wexler, S.; Burstain, I. G.; Denny, R.; Schenck, G. O.; Schulte-Elte, K.-H *Tetrahedron* **1967**, *23*, 2583-2599. (b) Miura, M.; Nojima, M.; Kusabayashi, S. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2909–2913. (c) Feringa, B. L.; Butselaar, R. J. *Tetrahedron Lett.* **1983**, *24*, 1193–1196. (d) McCullough, K. J.; Nojima, M.; Miura, M.; Jujisaka, T.; Kusabayashi, S. *J. Chem. Soc., Chem. Commun.* **1984**, *35*–37.

(23) The alternative hydroperoxide structure may be rejected on other chemical grounds as well. Thus, **4a** does not give a positive lead tetraacetate test for hydroperoxides according to Criegee et al. (Criegee, R.; Pilz, H.; Flygare, H. *Ber.* **1939**, *72B*, 1799–1804) nor does **4a** form a perester as is the case for hydroperoxy ethers and hydroperoxy epidioxides, cf. Milas, N. A.; Golubović, A. *J. Org. Chem.* **1962**, *27*, 4319–4323.

(24) Intermolecular associations of **4**, **6**, and **6** 3β-acetates also are evident from the dilution-sensitive 3450 cm^{-1} bands in their spectra. Intermolecular associations of **4a** are further indicated by results of cryoscopic determinations of molecular weight of **4a** in benzene, where apparent values of 1310 (14.4 mg/mL) and 1050 (16.0 mg/mL) were obtained (calcd. M_r 821.35).

yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (**9**). Proton and carbon spectra of **8** and **9** exhibit details of both the secosterol moiety of **4a** or **6** and 1,2,4-trioxolanes **2** and **3** and permit assignment of the 5′α,7′α-epidioxide ozonide feature of **8** to the more mobile dimer ozonide and the 5′β,7′β-epidioxide feature of **9** to the less mobile isomer in the same fashion used for assignment of stereochemistry for monomer ozonides **2** and **3**.

Three other mobile minor products were also recognized by proton spectra as mixtures of isomeric 1,2,4-trioxolane ozonides but of oligomeric species. Thus, broadened C-19 methyl proton signals, two distinct deshielded signals of hydrogen-bonded 5ξ-hydroxyl groups (at δ 10.18 and 10.3), and broad ($W_{1/2}$ = 18–21 Hz) signals at δ 5.5 of the 7α- and 7β-hydrogens of the isomeric 1,2,4-trioxolane ozonide features found in **2**, **3**, **8**, and **9** support these identifications. From the ratios of integrated signal intensities of the 6ξ-hydrogen (δ 4.8) and 7′ξ-hydrogen (δ 5.5) signals of the 5,6-epidioxy-5,6-secosterol and 1,2,4-trioxolane ozonide moieties respectively these minor products were recognized as trimeric, tetrameric, and pentameric analogues **10–12** of dimers **8** and **9** eluted from chromatograms in the decreasing order of their molecular weights.

Another pair of isomeric dimers formed from cholesterol ozonization were the epoxides 6ξ-(5′,6′α-epoxy-5′α-cholestan-3′β-yloxy)-5ξ-5,6-secocholestane-3β,5-diol (**13**) and 6ξ-(5′,6′β-epoxy-5′β-cholestan-3′β-yloxy)-5ξ-5,6-secocholestane-3β,5-diol (**14**). The 5′β,6′β-epoxide **14** was recovered directly by chromatography, and both **13** 3β-acetate and **14** 3β-acetate were recovered by chromatography of acetylated cholesterol ozonization products in approximately equal amounts, thus different from the 1:8 ratio of epoxides 5,6α-epoxy-5α-cholestan-3β-ol (**15**) and 5,6β-epoxy-5β-cholestan-3β-ol (**16**) formed during ozonization of cholesterol in alcoholic solvents.[5] Structures of the 5,6-epoxides were assigned from the unique one-proton signals at δ 2.89 (d, J = 4.1 Hz) of the 6′β-proton of a 5′α,6′α-epoxide feature in spectra of **13** 3β-acetate and at δ 3.05–3.06 of the 6′α-proton of a 5′β,6′β-epoxide feature in the spectra of **14** and **14** 3β-acetate, in analogy to similar signals in spectra of cholesterol 5,6-epoxides.[25] The structure of **13** 3β-acetate was confirmed by independent syntheses by epoxidation of **4b** with *m*-chloroperbenzoic acid and by ozonization of **1b** in the presence of an excess of the 5α,6α-epoxide **15**.

Neither epoxide **15** nor **16** was detected among products of cholesterol ozonization in nonparticipating solvents. However, the presence among products of the epoxides **13** and **14** suggests that epoxides **15** and **16** may form but be intercepted by carbonyl oxide **7**. This formulation is supported by our synthesis of **13** 3β-acetate from **1b** and **15** under ozonization conditions. It is likely that the absence of ozonides **2** and **3** but presence of **8** and **9** among products of cholesterol ozonization represents the related process of interception of **2** and **3** by **7**.

Yet other minor ozonization products were detected but not identified. The most mobile material (t_R 8.6 min) and the least mobile component (t_R 3.2 min) eluted just ahead of cholesterol were recognized as mixtures of related dimers from proton spectra (two C-19 signals and one low field sharp singlet), but six signals in the δ 2.6–5.5 range in each fraction could not be interpreted beyond that.

Discussion

These results establish that the reaction of cholesterol with O_3 in nonparticipating solvents involves the same two processes that occur in participating hydroxylic solvents, namely carbonyl oxide **7** formation and 5,6-epoxidation.[26] Two subsequent reactions of **7** then ensue, the predominant intermolecular interception of hydroxylic species whether solvent or sterol by **7** to form alkoxy epidioxides or the intramolecular interception of the 5-ketone feature of **7** by the 6-carbonyl oxide moiety to yield isomeric 1,2,4-trioxolane ozonides **2** and **3**. The initial, major olefinic product **4a** may then react with O_3 in the same manner, yielding isomeric 1,2,4-trioxolane ozonides **8** and **9** via 5′,6′ξ-epidioxy-3′β,5′-dihydroxy-5′ξ-(5′,6′-secocholestan-6′ξ-yloxy)-5-oxo-5,6-secocholestane-6-carbonyl oxide putatively formed and isomeric 5,6-epoxides **13** and **14**.

However, more than one process is implicated in formation of dimer ozonides **8** and **9** and dimer epoxides **13** and **14**, as interceptions by **7** of **2** and **3** yielding **8** and **9**, respectively, and of **15** and **16** yielding **13** and **14**, respectively, may occur, thereby accounting for the absence of **2**, **3**, **15**, and **16** from cholesterol ozonization products.

Derivation of trimer **10** by interception of dimer **4a** by **7** followed by further reaction with O_3 is likely, but interceptions of **8** and **9** by **7** also may account for **10** formation. Even more complex schemes may be posed for derivation of the higher oligomers **11** and **12**. The complexity of these product mixtures ensures that no prior study of cholesterol ozonization ever dealt with single or pure products.

The chemistry of oligomer formation in ozonizations is still not understood.[8,27] Although our work is confined to reactions of O_3 with cholesterol as olefin and alcohol yielding a complex spate of dimeric and oligomeric products, one may posit that similar transformations be implicated in oligomer formation in other ozonizations. As there appears to be a continuum of reactivity of the hydroxyl group of lower alcohols with carbonyl oxides,[20] with cholesterol even less reactive and the hydrogen-bonded 5ξ-hydroxyl of **4** and **6** unreactive, it may be that carbonyl oxide interceptions of yet less reactive hydrogen from olefins occur, leading to epidioxy alcohol oligomers.[28]

(25) The 6-proton signals of the isomeric cholesterol 5,6-epoxides and 3β-esters are observed as doublets at δ 2.85–2.91 (J = 3.6-4 Hz) for the 6β-proton of 5α,6α-epoxide **15** and its esters and at δ 3.05-3.07 (J = 2-3 Hz) for the 6α-proton of 5β,6β-epoxide **16** and its esters; cf. (a) Tori, K.; Komeno, T.; Nakagawa, T. *J. Org. Chem.* **1964**, *29*, 1136–1141. (b) Lindgren, B. O.; Suahn, C. M. *Acta Chem. Scand.* **1970**, *24*, 2699–2704. (c) Assman, G.; Fredrickson, D. S.; Sloan, B. R.; Fales, H. M.; Highet, R. J. *J. Lipid Res.* **1975**, *16*, 28–38. (d) Ansari, G. A. S.; Smith, L. L. *Photochem. Photobiol.* **1979**, *30*, 147–150. (e) Tsai, L.-S.; Hudson, C. A. *J. Food Sci.* **1984**, *49*, 1245–1248. (f) Raaphorst, G. P.; Azzam, E. I.; Langlois, R.; van Lier, J. E. *Biochem. Pharmacol.* **1987**, *36*, 2369–2372.

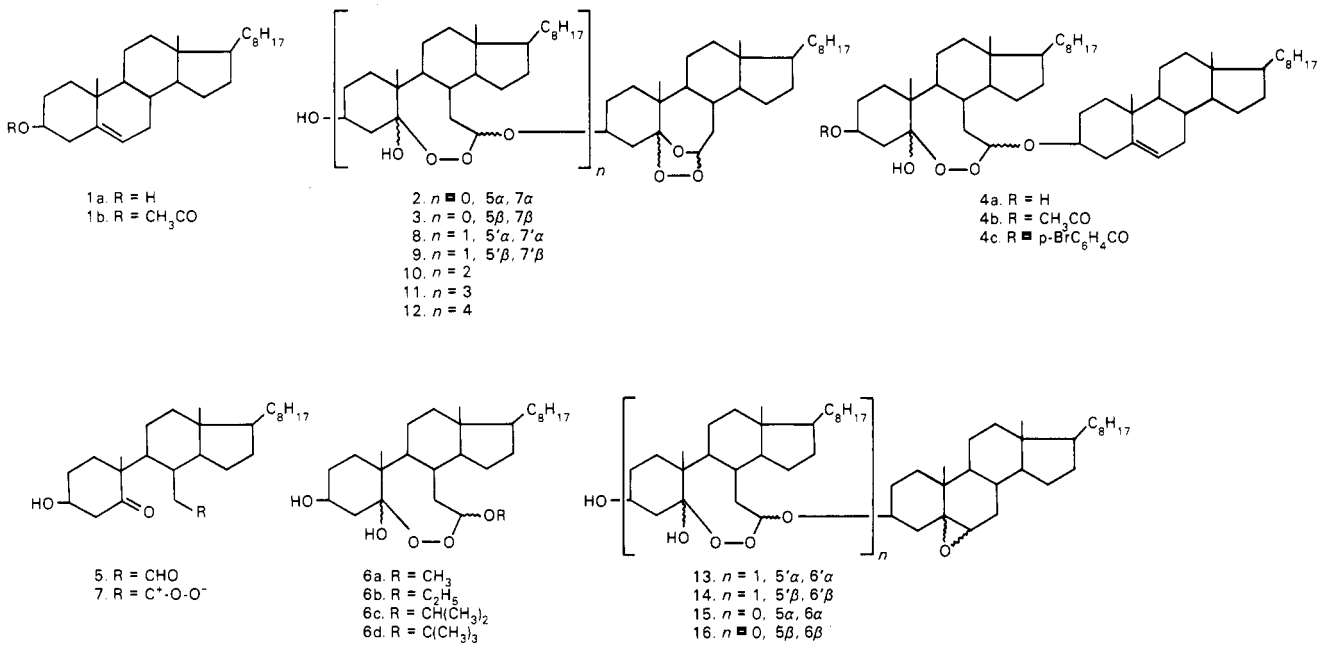
(26) Carbonyl oxide formation and epoxidation are recognized as separate reactions occurring during ozonization of olefins; cf. (a) Sawacki, Y.; Kato, H.; Ogata, Y. *J. Am. Chem. Soc.* **1981**, *103*, 3832–3837. (b) Bailey, P. S.; Hwang, H. H.; Chiang, C.-Y. *J. Org. Chem.* **1985**, *50*, 231–234.

The alternative epoxidation of cholesterol not by O_3 but by carbonyl oxide **7** or regioisomeric 6-oxo 5-carbonyl oxide (putatively formed to a less extent than **7** along lines suggested by Hinrichs et al. (Hinrichs, T. A.; Ramachandran, V.; Murray, R. W. *J. Am. Chem. Soc.* **1979**, *101*, 1282–1284) is not indicated as such a process requires formation of an equivalent amount of **5** (or of dimeric seco aldehyde 3β-5′,6′ξ-epidioxy-3′β,5′-dihydroxy-5′ξ-(5′,6′-secocholestan-6′ξ-yloxy)-5-oxo-5,6-secocholestan-6-al, neither being found among cholesterol ozonization products.

(27) Bailey, P. S. *Ozonization in Organic Chemistry*; Academic Press: New York, 1978; Vol. I, 33–37, pp 83–87.

(28) Hydroxyl (3425 cm^{-1}) absorption has been noted in spectra of an oligomer from ozonization of *trans*-hex-3-ene, cf. ref 7.

Chart I



Experimental Section[29]

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_3–O_2 stream (containing approximately 0.18 mequiv O_3/min) was passed through the solution to be oxidized, with aliquots periodically analyzed by thin-layer chromatography and HPLC to monitor progress of the ozonization.

5,7α-Epidioxy-5α-*B*-homo-6-oxacholestan-3β-ol (2). A solution of 42 mg of cholesterol in 50 mL of hexane was ozonized at room temperature for 5 min, the hexane was removed under vacuum, and the oily material was subjected to HPLC in system G. Of the two major products, the more mobile (t_R 19.4 min) yielded 8.4 mg of pure **2**, crystallized from hexane–acetone: mp 110–115 °C; R_f 0.31 (system V); IR (KBr) 3420, 1213, 1194, 1178, 1148, 1125, 1105, 1095, 1055, 1034, 973, 950, 873, 832, 766, 705, 668, 590, 490 cm^{-1}; IR (CCl_4) 3640, 3500 (disappearing on dilution) cm^{-1}; NMR δ 0.66 (3 H, s, 18-H), 1.12 (3 H, s, 19-H), 2.24 (1 H, ddd, J = 3.7, 7.5, 15.1 Hz, 7aα-H), 3.83 (1 H, m, $W_{1/2}$ = 25 Hz, 3α-H), 5.54 (1 H, d, J = 6.8 Hz, 7β-H); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 434 (1.5), 416 (26), 401 (13), 372 (12), 317 (100), 249 (64), 247 (90); CI mass spectrum, m/z (relative intensity) 435 (90), 419 (22), 417 (100), 407 (22), 389 (20), 371 (15), 369 (10), 263 (6), 261 (6).

5,7α-Epidioxy-5α-*B*-homo-6-oxacholestan-3β-ol (2) 3β-Acetate. A solution of 591 mg of **1b** in 51 mL of CCl_4 was ozonized at room temperature for 60 min. Removal of solvent under vacuum gave 668 mg of an oily mixture of two major products, and HPLC of 450 mg in system E, with rechromatography in system A at 3–8 mL/min flow rate with recycling of major components ultimately gave 70 mg of pure **2** 3β-acetate crystallized from hexane: mp 91–95 °C; t_R 24.6 min in system B at 1.5 mL/min; R_f 0.29 (system IV); IR (KBr) 1750, 1260, 1198, 1178, 1140, 1127, 1083, 1038, 1010, 987, 970, 953, 873, 825, 798, 764, 700, 610, 585, 562, 550, 513, 490 cm^{-1}; NMR δ 0.62 (3 H, s, 18-H), 1.09 (3 H, s, 19-H), 1.99 (3 H, s, CH_3CO), 2.21 (1 H, ddd, J = 3.8, 8.0, 15.2 Hz, 7aα-H), 4.83 (1 H, m, $W_{1/2}$ = 24 Hz, 3α-H), 5.49 (1 H, d, J = 6.8 Hz, 7β-H); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 416 (1), 401 (0.5), 398 (1), 357 (0.5), 331 (1), 317 (3), 275 (2), 249 (4), 143 (100); CI mass spectrum, m/z (relative intensity) 475 (1), 461 (1), 459 (16), 417 (20), 399 (100), 389 (12), 373 (13), 371 (13), 357 (14). Anal. Calcd for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15; O, 16.78. Found: C, 72.89; H, 10.15; O, 17.03.

Acetylation of 17 mg of **2** in 1 mL of pyridine and 0.3 mL of acetic anhydride yielded two more polar components, neither of which corresponded to **2** 3β-acetate derived from **1b** not further investigated.

5,7β-Epidioxy-5β-*B*-homo-6-oxacholestan-3β-ol (3). The second less mobile fraction (t_R 20.8 min, system G) from the ozonization of cholesterol in hexane yielded 5.6 mg of pure **3** crystallized from benzene–ethyl acetate: mp 130–132 °C; R_f 0.29 (system V); IR (KBr) 3400, 1197, 1175, 1158, 1142, 1120, 1090,

(29) Melting points were taken on a Kofler block under a microscope. Thin-layer chromatography was conducted using 8-cm-long silica gel chromatoplates No. 5554 (E. Merck GmbH., Darmstadt) irrigated with systems I, benzene; II, benzene–hexane (1:1, v/v); III, benzene–ethyl acetate (10:1, v/v); VI, benzene–ethyl acetate (20:1, v/v) and with 10-cm-long No. 13727 HPLTC silica gel chromatoplates with concentrating zone (E. Merck GmbH.) irrigated with systems IV, benzene; V, benzene–ethyl acetate (10:1, v/v). Sterols were detected by examination under 254 nm light, by spraying with *N,N*-dimethyl-*p*-phenylenediamine to detect peroxides (cf. Smith, L. L.; Hill, F. L. *J. Chromatogr.* **1972**, *66*, 101–109), and by spraying with 50% aqueous sulfuric acid (with heating or charring) to detect all sterols. Brown colors were uniformly obtained with these secosterols.

High performance liquid chromatography was conducted by using equipment of Waters Associates, Milford, MA, and a Perkin-Elmer Corp. Model LC-55 variable wavelength spectrophotometric detector set at 212 nm and with the Waters Associates Prep LC/System 500 preparative HPLC equipment with detection of eluted components by differential refractive index measurements using the Model R4-1 detector. Binary solvent mixtures were used for irrigations of three different HPLC columns in the following arrangements: A 10 mm × 25 cm 5 μm particulate silica column (IBM Instruments, Wallingford, CT) with system A, hexane–isopropyl alcohol (600:1, v/v) at 3.0, 5.0, or 8.0 mL/min; system B, hexane–isopropyl alcohol (480:1, v/v) at 1.5 or 2.5 mL/min; system C, distilled hexane–isopropyl alcohol (480:1, v/v)–isopropyl alcohol (1000:1, v/v) at 3.0 mL/min; system D, benzene–ethyl acetate (100:1, v/v) at 3.0 mL/min and two such columns in tandem with system I, benzene–ethyl acetate (50:3, v/v) at 3.0 mL/min; system J, hexane–isopropyl alcohol (240:1, v/v) at 3.0 mL/min; and K, distilled benzene–ethyl acetate (25:1, v/v) at 3.0 mL/min. A 25.4 mm × 30 cm 37–55 μm particulate silica column (Waters Associates) with system E, hexane–isopropyl alcohol (120:1, v/v) at 6.0 mL/min and two such columns in tandem with system F, benzene–ethyl acetate (25:3, v/v) at 7.0 mL/min. Two 7.8 mm × 30 cm 10 μm particulate μ-Porasil (Waters Associates) columns in tandem with system G, benzene–ethyl acetate (25:3, v/v) at 3.0 mL/min; system H, benzene–ethyl acetate (5:2, v/v) at 3.0 mL/min.

Mobility data characterizing each sterol and expressed as R_f (thin-layer) and t_R (retention time, HPLC).

Proton and carbon spectra were recorded on deuteriochloroform solutions of sterols on Nicolet 300-MHz and JEOL 270-MHz spectrometers, with chemical shifts reported with tetramethylsilane as internal reference. Infrared absorption spectra over the range 400-4000 cm^{-1} were obtained with a Perkin-Elmer Model 337 spectrometer equipped with a beam condenser, using 0.1 mm KBr disks incorporating the sample or CCl_4 solutions. Mass spectra were obtained on samples directly introduced by probe into the ionization region of a Finnigan Corp. Model 4000 quadrupole mass spectrometer. Electron impact mass spectra were obtained at 70 eV; CI mass spectra were obtained using methane as reagent gas. Elemental analyses and cryoscopic (benzene as solvent) molecular weights were by Huffman Laboratories Inc., Wheat Ridge, CO.

1060, 1030, 1020, 980, 958, 855, 710, 668, 587, 490 cm^{-1}; IR (CCl_4) 3640, 3500 and 3400 (both disappearing on dilution) cm^{-1}; NMR δ 0.68 (3 H, s, 18-H), 1.01 (3 H, s, 19-H), 2.08 (1 H, d, J = 5.9 Hz, 7aα-H), 3.82 (1 H, m, $W_{1/2}$ = 22 Hz, 3α-H), 5.57 (1 H, d, J = 1.5 Hz, 7α-H); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 434 (2), 416 (7), 401 (6), 372 (6), 317 (80), 303 (27), 275 (35), 262 (100), 247 (96); CI mass spectrum, m/z (relative intensity) 435 (75), 419 (20), 417 (100), 407 (12), 389 (21), 373 (17), 331 (12), 261 (10).

Repetition of the ozonization under the same conditions except at much greater CCl_4 dilutions (4.6 mg of cholesterol/51 mL of CCl_4) for 30 s and in hexane or in heptane under the same conditions gave after chromatography in system G 1.7 mg of **2**, t_R 19.4 min, and 0.8 mg of **3**, t_R 20.9 min.

5,7β-Epidioxy-5β-*B*-homo-6-oxacholestan-3β-ol (3) 3β-Acetate. A. From 1b. The second more mobile component (R_f 0.33, system IV; t_R 23.9 min, system B) from the ozonization of **1b** was crystallized from hexane, 36 mg: mp 123–125 °C; IR (KBr) 1755, 1252, 1203, 1175, 1155, 1138, 1112, 1067, 1060, 1034, 1013, 998, 977, 956, 856, 668, 614, 602, 580, 570, 516, 490 cm^{-1}; NMR δ 0.63 (3 H, s, 18-H), 0.99 (3 H, s, 19-H), 1.99 (3 H, s, CH_3CO), 2.11 (1 H, dd, J = 1.9, 5.6 Hz, 7aα-H), 4.78 (1 H, m, $W_{1/2}$ = 23 Hz, 3α-H), 5.55 (1 H, d, J = 1.7 Hz, 7α-H); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 416 (0.4), 401 (0.5), 331 (0.4), 317 (2.5), 275 (2), 249 (2.5), 247 (3), 143 (100); CI mass spectrum, m/z (relative intensity) 475 (1), 459 (15), 417 (15), 399 (100), 387 (10), 371 (13). Anal. Calcd for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15; O, 16.78. Found: C, 72.86; H, 10.24; O, 17.21.

B. From 3. To a solution of 7.5 mg of **3** in 0.5 mL of pyridine was added 0.2 mL of acetic anhydride. After 22 h at room temperature the solution was poured onto ice, and the product was recovered as previously described. Pure **3** 3β-acetate (3 mg) was obtained following HPLC in system A at 5.0 mL/min and crystallization from hexane, mp 122–125 °C, identical in infrared, proton, and carbon spectra and in chromatographic properties with the sample derived from **1b**.

6ξ-(Cholest-5′-en-3β′-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (4a). A solution of 1.010 g of cholesterol in 51 mL of CCl_4 was ozonized at room temperature for 15 min, by which time much of the cholesterol had been consumed. Solvent was removed under vacuum at 38 °C, yielding an oily residue composed of at least ten components more mobile than cholesterol characterized in HPLC systems G and I: t_R 6.6 and 12.6 min, unidentified most mobile oligomers; t_R 6.8 and 12.8 min, unidentified oligomers; t_R 7.9 and 13.4 min, pentamer **12**; t_R 8.1 and 13.9 min, tetramer **11**; t_R 8.4 and 14.8 min, trimer **10**; t_R 8.8 and 15.6 min, dimer **4a**; t_R 9.5 and 16.1 min, dimer ozonide **8**; t_R 9.5 and 16.6 min, dimer ozonide **9**; t_R 10.0 and 18.2 min, dimer epoxide **14**; t_R 13.2 min (system G), unidentified material.

Preparative HPLC in system F gave four fractions: t_R 23–33 min, 64 mg, most mobile materials and oligomers **10–12**; t_R 33–42 min, 309 mg of **4a**; t_R 42–67 min, 137 mg of **4a**, **8**, **9**, **13**, **14**, and unidentified least mobile material; and t_R 67–92 min, 402 mg of unreacted cholesterol. The t_R 33–42 min fraction containing **4a** was rechromatographed on system D, yielding pure **4a** with t_R 16.4 min as an amorphous solid: mp 133–136 °C; R_f 0.50 (system III); IR (KBr) 3440, 3312, 1148, 1070, 1045, 1012, 996, 978, 960, 952 cm^{-1}; IR (CCl_4) 3640, 3425, 3320 cm^{-1}; NMR δ 0.65 (3 H, s, 18′-H), 0.67 (3 H, s, 18-H), 0.99 (3 H, s, 19′-H), 1.04 (3 H, s, 19-H), 2.34 (1 H, dd, J = 5.6, 13.0 Hz, 4′α-H), 2.61 (1 H, d, J = 11.7 Hz, 4α-H), 3.66 (1 H, m, $W_{1/2}$ = 23 Hz, 3′α-H), 3.87 (1 H, m, $W_{1/2}$ = 22 Hz, 3α-H), 4.89 (1 H, dd, J = 6.2, 9.7 Hz, 6ξ-H), 5.34 (1 H, d, J = 5.1 Hz, 6′-H), 10.38 (1 H, s, 5ξ-OH); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 402 (3), 400 (5), 386 (96), 385 (7), 371 (40), 370 (50), 368 (44), 353 (26), 301 (46), 275 (62), 273 (37), 257 (20), 255 (26), 231 (36), 213 (40), 107 (100); CI mass spectrum, m/z (relative intensity) 417 (2), 415 (2), 413 (3), 401 (3), 399 (3), 387 (1), 386 (15), 385 (44), 371 (28), 370 (35), 369 (100), 367 (24), 355 (14). Anal. Calcd for $C_{54}H_{92}O_5$: C, 78.97; H, 11.29; O, 9.74; M_r 821.32. Found: C, 78.59: H, 11.08; O, 10.77; M_r 1310, 1050.[24]

3β-Acetoxy-6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (4b). A. From 4a. To a solution of 600 mg of **4a** in 9.5 mL of pyridine was added 2.5 mL of acetic anhydride. After 24 h at room temperature the solution was poured onto ice (containing NaCl) and the mixture was extracted with benzene–ethyl ether (1:1, v/v). The organic layer was washed with saturated $NaHCO_3$ solution twice and with water, dried over anhydrous Na_2SO_4, and evaporated under vacuum, yielding 660 mg of oily product. Chromatography in system B at 2.5 mL/min with rechromatography in system C gave 72.8 mg of **4b** with t_R 13.2 min, R_f 0.40 (system I), and 25.1 mg of four more mobile compounds not further examined. Crystallization from hexane–acetone gave pure crystalline **4b**: mp 164–167 °C; IR (KBr) 3310, 1740, 1148, 1063, 1040, 1013, 994, 980, 960 cm^{-1}; IR (CCl_4) 3320, 1740 cm^{-1}; NMR δ 0.63 (3 H, s, 18′-H), 0.65 (3 H, s, 18-H), 0.96 (3 H, s, 19′-H), 1.02 (3 H, s, 19-H), 2.00 (3 H, s, CH_3CO), 2.30 (1 H, dd, J = 4.7, 12.8 Hz, 4′α-H), 2.58 (1 H, d, J = 12.8 Hz, 4α-H), 3.60 (1 H, m, $W_{1/2}$ = 24 Hz, 3′α-H), 4.86 (1 H, m, 3α-H), 4.86 (1 H, dd, J = 5.5, 9.8 Hz, 6ξ-H), 5.31 (1 H, d, J = 5.8 Hz, 6′-H), 10.29 (1 H, s, 5ξ-OH); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 416 (0.5), 400 (0.4), 386 (6.5), 385 (0.5), 384 (2), 370 (7), 368 (6.5), 353 (4), 301 (12), 275 (20), 273 (7), 257 (12), 255 (12), 231 (12), 215 (26), 213 (22), 95 (100); CI mass spectrum, m/z (relative intensity) 475 (0.8), 459 (7), 427 (2.4), 415 (7), 399 (60), 389 (28), 383 (9), 373 (19), 371 (22), 369 (5), 355 (26), 353 (12), 305 (21), 275 (19), 263 (33), 261 (58), 143 (100). Anal. Calcd for $C_{56}H_{94}O_6$: C, 77.91: H, 10.97; O, 11.12; M_r, 863.36. Found: C, 77.37; H, 10.78; O, 11.66; M_r, 770.

B. From Acetylated Mixed Products. A solution of 38 mg of total ozonization products of cholesterol in 2 mL of dry pyridine treated with 0.5 mL of acetic anhydride gave after HPLC 3 mg of **4b** identical in spectral and chromatographic properties with **4a** prepared in A above.

3β-[(*p*-Bromobenzoyl)oxy]-6ξ-(cholest-5′-en-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (4c). To a solution of 80 mg of **4a** in 1 mL of CH_2Cl_2 cooled to 0 °C was added a solution of *p*-bromobenzoyl trifluoromethanesulfonate (prepared by adding 1 mL of CH_2Cl_2 and 0.071 mL of pyridine to a solution of 72.4 mg of *p*-bromobenzoyl chloride in 0.031 mL of trifluoromethanesulfonic acid that had aged at room temperature for 21 h).[30] One hour after the reaction mixture had warmed to room temperature, it was poured into water, and products were extracted with benzene–diethyl ether (1:1, v/v). The organic layer was washed with saturated $NaHCO_3$ solution twice, with 1 M H_2SO_4, and with water, then dried over anhydrous Na_2SO_4, and evaporated under vacuum. Chromatography on 230–400-mesh silica gel with hexane–benzene (1:1, v/v) and crystallization of the ester from ethyl acetate gave 47 mg of pure **4c**: mp 138–140 °C; R_f 0.4 (system II); IR (KBr) 3320, 1730, 1600, 1285, 1182, 1147, 1123, 1110, 1077, 1038, 1017, 995, 980, 960, 850, 760, 684, 628 cm^{-1}; IR (CCl_4) 3320, 1730 cm^{-1}; NMR δ 0.66 (6 H, s, 18-H, 18′-H), 0.98 (3 H, s, 19′-H), 1.09 (3 H, s, 19-H), 2.34 (1 H, dd, J = 4.2, 12.8 Hz, 4′α-H), 2.75 (1 H, dd, J = 4.6, 12.8 Hz, 4α-H), 3.65 (1 H, m, $W_{1/2}$ = 24 Hz, 3′α-H), 4.91 (1 H, dd, J = 5.7, 9.8 Hz, 6ξ-H), 5.15 (1 H, m, $W_{1/2}$ = 24 Hz, 3α-H), 5.34 (1 H, d, J = 4.7 Hz, 6′-H), 7.56 (2 H, d, J = 8.1 Hz, aromatic H), 7.90 (2 H, d, J = 8.1 Hz, aromatic H), 10.35 (1 H, s, 5ξ-OH); EI mass spectrum, m/z (relative intensity) 472 (2), 430 (11), 386 (5), 368 (8), 353 (3), 301 (5), 275 (7), 255 (5), 247 (7), 213 (8), 207 (12), 183 (33), 149 (56), 125 (50), 111 (100); CI mass spectrum, m/z (relative intensity) 457 (2), 445 (3), 431 (3), 427 (5), 417 (27), 401 (15), 399 (43), 387 (16), 385 (41), 369 (100), 331 (8). Anal. Calcd for $C_{61}H_{95}O_6Br$: C, 72.95; H, 9.53; O, 9.56; Br, 7.96; M_r, 1004.33. Found: C, 72.74; H, 9.45; O, 10.38; Br, 8.22; M_r, 995.

3β-Hydroxy-5-oxo-5,6-secocholestan-6-al (5). To a suspension of 10 mg of **4a** in 12 mL of acetic acid was added to 10 mg of Zn dust. The mixture was occasionally shaken and allowed to stand at room temperature overnight. Sterols were recovered by extraction with benzene–diethyl ether, the extracts being washed with $NaHCO_3$ solution and with water, dried over anhydrous Na_2SO_4, and evaporated under vacuum. Following HPLC in system H there was obtained 3.0 mg of pure **5** as an oil (IR (KBr) 3450 (OH), 2730 (CHO), 1725 (CHO), 1710 (CO) cm^{-1} (lit. IR (CCl_4) 3650, 3450, 2730, 1730, 1710 cm^{-1}, ref 5)) and 4.0 mg of cholesterol both identified by comparison of chromatographic

(30) Brown, L.; Koreeda, M. *J. Org. Chem.* **1984**, *49*, 3875–3880.
(31) (a) Tanabe, K.; Hayashi, R.; Takasaki, R. *Chem. Pharm. Bull.* **1961**, *9*, 1–6. (b) Buckingham, J.; Chittenden, G. J. F.; Guthrie, R. D. *J. Chem. Soc.* **1967**, 1703–1706. (c) Morand, P.; Kaufman, M. *J. Org. Chem.* **1969**, *34*, 2175–2180.

and spectral properties with authentic samples. The same 6-aldehyde **5** and cholesterol were obtained from the reduction of **4a** in methanol with $(CH_3)_2S$; from reduction of **2** and **3** with Zn/acetic acid **5** was also obtained.

3β-Acetoxy-5-oxo-5,6-secocholestan-6-al (5 3β-Acetate). A. From 4b. A suspension of 10 mg of **4b** in 12 mL of acetic acid treated with Zn dust in the same manner gave after HPLC in system G 3.5 mg of **5** 3β-acetate as an amorphous solid (IR (KBr) 2730 (CHO), 1725 (CHO, CH_3COO), 1710 (CO) cm^{-1} (lit. IR (Nujol) 2688, 1739, 1704 cm^{-1}; IR 2705, 1740, 1705 cm^{-1}; IR ($CHCl_3$) 2715, 1735, 1725, 1700 cm^{-1}; ref 31)) and 4 mg of cholesterol, both identified by chromatographic and spectral comparisons with authentic samples.

B. From 2 3β-Acetate and from 3 3β-Acetate. Reduction in the same manner of 14.5 mg of **2** 3β-acetate in 1.9 mL of acetic acid with 44 mg of Zn dust gave 14.0 mg of oily material that afforded 5.0 mg of **5** 3β-acetate following HPLC in system G. Similar reduction of 8 mg of **3** 3β-acetate gave 6.5 mg of oily material that yielded 3.2 mg of **5** 3β-acetate following HPLC in system G. All samples of **5** 3β-acetate were identical by spectral and chromatographic data.

6ξ-5′,7′α-Epidioxy-5′α-(*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (8). A. From Cholesterol. Rechromatography of the t_R 42–67 min component from cholesterol ozonization in system G and then in system D yielded pure **8** as an amorphous solid: mp 118–120 °C; t_R 19.8 min; R_f 0.50 (system III); IR (KBr) 3450, 3320, 1177, 1150, 1085, 1070, 1050, 1037, 1010, 977, 960 cm^{-1}; IR (CCl_4) 3640, 3325, cm^{-1}; NMR δ 0.64 (3 H, s, 18-H), 0.65 (3 H, s, 18′-H), 1.04 (3 H, s, 19-H), 1.10 (3 H, s, 19′-H), 2.24 (1 H, ddd, J = 3.7, 7.7, 15.2 Hz, 7aα-H), 2.61 (1 H, d, J = 12.7 Hz, 4α-H), 3.87 (1 H, m, $W_{1/2}$ = 24 Hz, 3′α-H), 3.88 (1 H, m, $W_{1/2}$ = 24 Hz, 3α-H), 4.86 (1 H, dd, J = 5.9, 9.8 Hz, 6ξ-H), 5.53 (1 H, d, J = 7.3 Hz, 7′β-H), 10.22 (1 H, s, 5ξ-OH); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 434 (4), 418 (3), 416 (8), 386 (7), 368 (2), 357 (7), 349 (8), 347 (10), 331 (14), 317 (57), 315 (34), 303 (25), 301 (28), 289 (26), 274 (58), 249 (100); CI mass spectrum, m/z (relative intensity) 435 (70), 419 (28), 417 (100), 405 (26), 399 (38), 389 (51), 369 (39), 331 (34), 279 (34). Anal. Calcd for $C_{54}H_{92}O_8$: C, 74.61; H, 10.67; O, 14.72; M_r, 869.32. Found: C, 74.04; H, 10.52; O, 15.68; M_r, 864.

B. From 4a. A solution of 130 mg of **4a** in 50 mL of CCl_4 was ozonized at room temperature for 10 min, at which time **4a** was consumed and thin-layer chromatography evinced formation of two major products (R_f 0.45 and 0.50 in system III). Following HPLC in system D 15 mg of pure **8**, mp 116–121 °C, t_R 19.7 min, was isolated as an amorphous solid, identical in infrared, proton, and carbon spectra and in chromatographic properties with **8** prepared from cholesterol.

3β-Acetoxy-6ξ-(5′,7′α-epidioxy-5′α-*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (8 3β-Acetate). A. From 8. To a solution of 19.9 mg of **8** in 1 mL of pyridine was added 0.5 mL of acetic anhydride. After 22 h at room temperature the solution was poured onto ice and salt, and the product was recovered as previously described. Pure **8** 3β-acetate (1.5 mg) was obtained following HPLC in system C, t_R 8.7 min, R_f 0.77 (system VI), and crystallization from hexane: mp 139–142 °C; IR (KBr) 3470, 3310, 1740, 1252, 1195, 1148, 1115, 1065, 1048, 1020, 977, 960, 927, 883, 870, 610 cm^{-1}; IR (CCl_4) 3310, 1735 cm^{-1}; NMR δ 0.64 (3 H, s, 18-H), 0.66 (3 H, s, 18′-H), 1.00 (3 H, s, 19-H), 1.05 (3 H, s, 19′-H), 2.03 (3 H, s, CH_3CO), 2.32 (1 H, m, 4′α-H), 2.61 (1 H, dd, J = 4.3, 13.7 Hz, 4α-H), 3.87 (1 H, m, $W_{1/2}$ = 26 Hz, 3α-H), 4.89 (1 H, dd, J = 4.7, 9.8 Hz, 6ξ-H), 4.91 (1 H, m, $W_{1/2}$ = 25 Hz, 3′α-H), 5.57 (1 H, br s, 7′β-H), 10.19 (1 H, s, 5ξ-OH).

B. From Acetylated Mixed Ozonization Products. A solution of 600 mg of total ozonization products of cholesterol in 9.5 mL of pyridine was treated with 2.5 mL of acetic anhydride and was processed as previously described. Pure **8** 3β-acetate (7.5 mg) identical in spectral and chromatographic properties with **8** 3β-acetate prepared under A was obtained by HPLC in system B at 2.5 mL/min and then in system C.

6ξ-(5′,7′β-Epidioxy-5′β-*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestane-3β,5-diol (9). A. From Cholesterol. Rechromatography on the t_R 42–67 min component from which the dimer 5α,7α-ozonide **8** was recovered also afforded the dimer 5β,7β-ozonide **9**, t_R 20.7 min, as an amorphous solid: mp 130–135 °C; R_f 0.45 (system III); IR (KBr) 3470, 3320, 1177, 1150, 1075, 1050, 1036, 1012, 985, 950 cm^{-1}; IR (CCl_4) 3645, 3320 cm^{-1}; NMR δ 0.64 (3 H, s, 18-H), 0.66 (3 H, s, 18′-H), 1.00 (3 H, s, 19-H), 1.04 (3 H, s, 19′-H), 2.11 (1 H, dd, J = 3.6, 11.9 Hz, 4′α-H), 2.58 (1 H, dd, J = 2.5, 12.8 Hz, 4α-H), 3.85 (2 H, m, $W_{1/2}$ = 26 Hz, 3α-H, 3′α-H), 4.87 (1 H, dd, J = 6.1, 9.8 Hz, 6ξ-H), 5.57 (1 H, d, J = 1.7 Hz, 7′α-H), 10.23 (1 H, s, 5ξ-OH); carbon spectrum, cf. Table I; EI mass spectrum, m/z (relative intensity) 434 (1), 416 (7), 375 (7), 358 (8), 331 (22), 318 (100), 301 (25), 289 (27), 249 (67), 247 (99); CI mass spectrum, m/z (relative intensity) 435 (16), 417 (100), 399 (38), 371 (16), 305 (7), 247 (4). Anal. Calcd for $C_{54}H_{92}O_5$: C, 74.61; H, 10.67; O, 14.72; M_r, 869.32. Found: C, 74.54; H, 10.67; O, 15.06; M_r, 1251.

B. From 4a. The second more polar component formed from **4a** was rechromatographed in the same manner, yielding 16 mg of **9** as an amorphous solid, mp 129–134 °C, t_R 20.6 min, identical in infrared, proton, and carbon spectra and in chromatographic properties with **9** prepared from cholesterol.

3β-Acetoxy-6ξ-5′,7′β-epidioxy-5′β-(*B*′-homo-6′-oxacholestan-3′β-yloxy)-5,6ξ-epidioxy-5ξ-5,6-secocholestan-5-ol (9 3β-Acetate). A. From 9. To a solution of 12.3 mg of **9** in 1 mL of pyridine was added 0.5 mL of acetic anhydride. After 20 h the solution was poured onto ice and the product recovered in the usual manner, yielding 1.3 mg of pure **9** 3β-acetate, t_R 9.5 min (system C), R_f 0.70 (system VI), crystallized from hexane: mp 142–144 °C; IR (KBr) 3450, 3310, 1740, 1250, 1218, 1197, 1178, 1146, 1113, 1065, 1050, 1010, 958, 950, 924, 881, 870, 836, 825, 800, 738, 610 cm^{-1}; IR (CCl_4) 3305, 1740 cm^{-1}; NMR δ 0.64 (3 H, s, 18-H), 0.66 (3 H, s, 18′-H), 1.05 (3 H, s, 19′-H), 1.11 (3 H, s, 19-H), 2.03 (3 H, s, CH_3CO), 2.61 (1 H, dd, J = 4.7, 12.8 Hz, 4α-H), 3.87 (1 H, m, $W_{1/2}$ = 26 Hz, 3α-H), 4.88 (1 H, dd, J = 4.3, 9.4 Hz, 6ξ-H), 4.91 (1 H, m, $W_{1/2}$ = 27 Hz, 3′α-H), 5.58 (1 H, s, 7′α-H), 10.19 (1 H, s, 5ξ-OH).

B. From Acetylated Mixed Ozonization Products. Mixed products (600 mg) of cholesterol ozonization were similarly acetylated and subjected to HPLC in systems B and C and afforded 4.5 mg of **9** 3β-acetate, identical in spectral and chromatographic properties with the sample obtained under A.

Oligomeric Ozonides. A solution of 1.008 g of cholesxterol in 50 mL of CCl_4 was ozonized for 60 min at room temperature, during which time 20 mL of CCl_4 was added. Removal of solvent under vacuum yielded an oily product, of which 122 mg was subjected to HPLC in system G. Nine components were resolved: t_R 6.4–7.1 min, most mobile materials; t_R 7.1–8.2 min, benzene and most mobile materials; t_R 8.4 min, most mobile materials, pentamer **12**, and tetramer **11**; t_R 8.8 min, tetramer **11** and trimer **10**; t_R 9.5 min, trimer **10**, **8**, and **9**; t_R 9.8–10.2 min, **8**, **9**, and **14**; t_R 13.2 min, unidentified least mobile material; t_R 18.3 min, **2**; t_R 19.5 min, **3**.

Trimer 10. The t_R 8.8 min and t_R 9.5 min components were combined and rechromatographed in system I, yielding 2.7 mg of **10** as amorphous solid, t_R 14.8 min: IR (KBr) 3450, 3425, 3310, 1308, 1248, 1230, 1212, 1195, 1177, 1148, 1110, 1070, 1050, 1005, 950, 923, 875, 829, 798, cm^{-1}; IR (CCl_4) 3640, 3310 cm^{-1}; NMR δ 0.66 (br), 1.05 (br), 2.59 (1 H, m, $W_{1/2}$ = 18 Hz), 3.88 (3 H, m, $W_{1/2}$ = 27 Hz), 4.87 (2 H, m, $W_{1/2}$ = 18 Hz), 5.55 (1 H, m, $W_{1/2}$ = 20 Hz), 10.18 (1 H, s, 5ξ-OH), 10.29 (1 H, s, 5′ξ-OH).

Tetramer 11. Rechromatography of the t_R 8.4 min and t_R 8.8 min components in system I gave 1.6 mg of **11**, t_R 13.9 min: IR (KBr) 3450, 3310, 1307, 1248, 1229, 1213, 1178, 1148, 1115, 1070, 1045, 1005, 955, 924, 879, 869, 826, 798, cm^{-1}; IR (CCl_4) 3640, 3310 cm^{-1}; NMR δ 0.62 (br), 1.00 (br), 1.10 (br), 2.57 (2 H, m, $W_{1/2}$ = 27 Hz), 3.86 (1 H, m, $W_{1/2}$ = 29 Hz), 4.86 (3 H, m, $W_{1/2}$ = 23 Hz), 5.55 (1 H, m, $W_{1/2}$ = 21 Hz), 10.18 (2 H, s, 5ξ-OH), 10.32 (1 H, s, 5′ξ-OH).

Pentamer 12. Rechromatography of the t_R 8.4 min component yielded 0.7 mg of **12**, t_R 13.4 min; IR (KBr) 3450, 3305, 1310, 1283, 1265, 1250, 1231, 1218, 1196, 1182, 1152, 1118, 1074, 1055, 1036, 1008, 960, 923, 882, 870, 830, 800 cm^{-1}; IR (CCl_4) 3640, 3300 cm^{-1}; NMR δ 0.65, 1.03, 1.15, 2.59 (3 H, m), 3.06, 3.88 (4 H, m, $W_{1/2}$ = 28 Hz), 4.88 (4 H, m, $W_{1/2}$ = 21 Hz), 5.57 (1 H, m, $W_{1/2}$ = 18

Hz), 10.18 (br s, 5ξ-OH), 10.32 (s, 5′ξ-OH).

3β-Acetoxy-5,6ξ-epidioxy-6ξ-(5′,6′α-epoxy-5′α-cholestan-3′β-yloxy)-5ξ-5,6-secocholestan-5-ol (13 3β-Acetate). A. From Acetylated Mixed Organization Products. Acetylation of 600 mg of mixed cholesterol ozonization products and HPLC in system B at 2.5 mL/min, then in system C, and in system J gave 4.2 mg of **13** 3β-acetate as an amorphous solid: mp 154–155 °C; t_R 16.1 min; R_f 0.58 (system VI); IR (KBr) 3450, 3315, 1745, 1305, 1250, 1192, 1178, 1145, 1113, 1062, 1020, 984, 959, 930, 908, 874, 819, 800, 750, 735, 668, 607, 561, 527 cm^{-1}; IR (CCl_4) 3320, 1740 cm^{-1}; NMR δ 0.60 (3 H, s, 18′-H), 0.64 (3 H, s, 18-H), 1.04 (6 H, s, 19-H, 19′-H), 2.03 (3 H, s, CH_3CO), 2.31 (1 H, dd, J = 7.8, 15.5 Hz, 4′α-H), 2.62 (1 H, dd, J = 3.1, 13.6 Hz, 4α-H), 2.89 (1 H, d, J = 4.1 Hz, 6′β-H), 3.96 (1 H, m, $W_{1/2}$ = 24 Hz, 3′α-H), 4.82 (1 H, dd, J = 5.9, 9.9 Hz, 6ξ-H), 4.88 (1 H, m, $W_{1/2}$ = 27 Hz, 3α-H), 10.25 (1 H, s, 5ξ-OH). Anal. Calcd for $C_{56}H_{94}O_7$: C, 76.49; H, 10.78; O, 12.73. Found: C, 76.48; H, 11.04; O, 12.76.

B. From 4b. A solution of 5 mg of **4b** in 1 mL of CCl_4 was treated with 3.6 mg of *m*-chloroperbenzoic acid at 7 °C for 14 h and then was poured onto ice and products were extracted with benzene–diethyl ether (1:1, v/v). The organic layer was washed with saturated $NaHCO_3$ solution three times, with 1 M H_2SO_4 twice, with half-saturated $(NH_4)_2SO_4$, and with water and was dried over anhydrous Na_2SO_4. After removal of solvent under vacuum, chromatography on silica gel (230–400 mesh) with benzene–ethyl acetate (40:1, v/v) and HPLC in system I yielded 2.8 mg of **13** 3β-acetate as an amorphous solid, mp 150–154 °C, identical in spectral and chromatographic properties with **13** 3β-acetate recovered from section A.

C. From 5,6α-Epoxy-5α-cholestan-3β-ol. A solution of 46 mg of cholesterol 3β-acetate and 474 mg of 5,6α-epoxy-5α-cholestan-3β-ol (**15**) in 50 mL of CCl_4 was ozonized for 10 min in the usual manner. After removal of solvent under vacuum and chromatography on 230–400-mesh silica gel with benzene–ethyl acetate (40:1, v/v) there was recovered a major fraction (165 mg) containing **13** 3β-acetate, which upon HPLC in system J yielded 69.2 mg of pure **13** 3β-acetate, mp 154–156 °C, identical in spectral and chromatographic properties with **13** 3β-acetate prepared in A and B.

5,6ξ-Epidioxy-6ξ-(5′,6′β-epoxy-5′β-cholestan-3′β-yloxy)-5ξ-5,6-secocholestane-3β,5-diol (14). Rechromatography in system I of the HPLC fractions from which **8** and **9** had been recovered yielded 2.0 mg of pure **14** as an amorphous solid, t_R 18.2 min: IR (KBr) 3450, 3320, 1250, 1230, 1214, 1177, 1150, 1115, 1072, 1050, 1013, 993, 980, 958, 924, 880, 825, 790 cm^{-1}; IR (CCl_4) 3640, 3315 cm^{-1}; NMR δ 0.63 (3 H, s, 18′-H), 0.64 (3 H, s, 18-H), 0.98 (3 H, s, 19′-H), 1.04 (3 H, s, 19-H), 2.04 (1 H, m, 4′α-H), 2.62 (1 H, d, J = 14.1 Hz, 4α-H), 3.05 (1 H, s, 6′α-H), 3.86 (2 H, m, 3α-H, 3′α-H), 4.83 (1 H, dd, J = 5.2, 9.9 Hz, 6ξ-H), 10.24 (1 H, s, 5ξ-OH).

3β-Acetoxy-5,6ξ-epidioxy-6ξ-(5′,6′-β-epoxy-5′β-cholestan-3′β-yloxy)-5ξ-5,6-secocholestan-5-ol (14 3β-Acetate). Following acetylation of 600 mg of total ozonization products of cholesterol as previously described and HPLC in system B at 2.5 mL/min, then in system C, and system J there was obtained 3.4 mg of **14** 3β-acetate as an amorphous solid: mp 171–173 °C; t_R 16.9 min; R_f 0.64 (system VI); IR (KBr) 3450, 3315, 1740, 1254, 1225, 1195, 1182, 1151, 1065, 1045, 1028, 1015, 996, 985, 963, 925, 883, 873, 823, 790, 670, 610 cm^{-1}; IR (CCl_4) 3320, 1740 cm^{-1}; NMR δ 0.63 (3 H, s, 18′-H), 0.64 (3 H, s, 18-H), 0.98 (3 H, s, 19′-H), 1.05 (3 H, s, 19-H), 2.04 (3 H, s, CH_3CO), 2.10 (1 H, m, 4′α-H), 2.63 (1 H, dd, J = 3.1, 13.7 Hz, 4α-H), 3.06 (1 H, s, 6′α-H), 3.81 (1 H, m, $W_{1/2}$ = 24 Hz, 3′α-H), 4.84 (1 H, dd, J = 5.8, 10.2 Hz, 6ξ-H), 4.90 (1 H, m, $W_{1/2}$ = 26 Hz, 3α-H), 10.22 (1 H, s, 5ξ-OH).

Unidentified Components. Material recovered from the t_R 12.6 min and 12.8 min fractions was rechromatographed in system K, yielding a component with t_R 8.6 min: IR (KBr) 3450, 3310, 1317, 1305, 1265, 1246, 1230, 1217, 1180, 1145, 1112, 1065, 1050, 1015, 955, 938, 878, 868, 837, 825, 796, 630, 607, 580, 565 cm^{-1}; IR (CCl_4) 3305 cm^{-1}; NMR δ 0.68 (6 H, s, 18-H, 18′-H), 1.05 and 1.10 (6 H, s, 19-H, 19′-H), 2.60 (1 H, m), 3.00 (1 H, d, J = 7.0 Hz), 4.07 (1 H, m), 5.08 (1 H, t, J = 7.0 Hz), 10.56 (1 H, s, 5ξ-OH).

Material recovered from the t_R 13.2 min component from HPLC in system G was a mixture of components not further investigated.

Chemistry of O-Silylated Ketene Acetals:[1] Stereocontrolled Synthesis of 2-Deoxy- and 2-Deoxy-2-*C*-alkyl-*erythro*-pentoses

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Diastereoselective aldol reactions of 2,3-*O*-isopropylidene-D-(and L-)-glyceraldehydes (D- and L-**2**) with ketene silyl acetal **1a** occurred in acetonitrile under mild conditions to give the corresponding *anti*-β-siloxy esters (D- and L-**3a**) as major products, which were converted to 2-deoxy-D-(and L-)-riboses through a few additional steps. The aldol reactions of D-**2** with α-monoalkyl-substituted ketene silyl acetals **1c–f** proceeded similarly to give all *anti*-α-alkyl-β-siloxy esters (**11a–14a**) as major products, which were converted into 2-deoxy-2-*C*-alkyl-*erythro*-pentoses (**17a,b**).

Although the aldol and the Michael reactions are important methods for carbon–carbon bond formation, these reactions with ester enolates are often complicated due to the occurrence of undesired side reactions. To overcome these problems, ketene silyl acetals are successfully used as the functional equivalents of ester enolates. Thus, ketene silyl acetals can react with carbonyl[2] and α,β-unsaturated carbonyl compounds[3] in the presence of Lewis acids [$TiCl_4$ and/or $Ti(i\text{-}PrO)_4$] to give the corresponding adducts in good yields. With Lewis acid, however, the synthetically useful O-silylated adducts could not be isolated. We have found that the use of acetonitrile as a solvent can greatly enhance the reactivity of ketene silyl acetals toward the α,β-unsaturated carbonyl compounds to give the corresponding O-silylated Michael adducts in

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