Autoxidation of Cholesterol *via* Hydroperoxide Intermediates[1]

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The autoxidation of cholesterol in air is shown to proceed both *via* the previously recognized photoinduced singlet oxygen attack on the A/B-ring system to give the well-known sequence of autoxidation products of cholesterol, and also by a biradical oxygen attack resulting in the formation of cholesterol 20α- and 25-hydroperoxides and their putative degradation products cholest-5-ene-3β,25-diol, cholest-5-ene-3β,20α-diol, chol-5-en-3β-ol, pregn-5-ene-3β,20α-diol, pregn-5-en-3β-ol, 3β-hydroxypregn-5-en-20-one, androst-5-ene-3β,17β-diol, androst-5-en-3β-ol, and 3β-hydroxyandrost-5-en-17-one.

As a phase of our interests in the autoxidation and metabolism of cholesterol,[2] we examined several large batches of commercial cholesterol for autoxidation products. The commonly encountered cholesterol autoxidation products 3β-hydroxycholest-5-en-7-one, cholesta-3,5-dien-7-one, cholest-5-ene-3β,7α-diol, cholest-5-ene-3β,7β-diol, and 5α-cholestane-3β,5,6β-triol were isolated or confidently detected.[3] We have established for the first time the presence in air-aged cholesterol of the putative primary product of the photoinduced singlet oxygen attack on cholesterol, 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide (**1a**),[4] and its rearrangement product, 3β-hydroxycholest-5-ene 7α-hydroperoxide (**2a**),[4d,5] from which are derived the several secondary autoxidation products mentioned above.[3d] Although titration and chromatographic data[2a,6] suggest the presence of sterol hydroperoxides in air-aged cholesterol, their specific presence in such samples has not previously been demonstrated.[7]

In addition to these autoxidation products and cholest-5-ene-3β,25-diol (**4a**),[9] we have isolated and characterized several new sterol hydroperoxides, seven known steroids not previously recognized as cholesterol autoxidation products, and chol-5-en-3β-ol (**7a**) from air-aged cholesterol. The identities of the seven known steroids cholest-5-ene-3β,20α-diol (**6a**), pregn-5-ene-3β,20α-diol (**9a**), pregn-5-en-3β-ol (**8a**), 3β-hydroxypregn-5-en-20-one (**10a**), androst-5-ene-3β,17β-diol (**12a**), androst-5-en-3β-ol (**11a**), and 3β-hydroxyandrost-5-en-17-one (**13a**) were established by direct comparisons of the steroid recovered from air-aged cholesterol with an authentic sample.

The structure of the cholane derivative **7a**, surmised from chromatographic and color-test behavior, infrared absorption spectra, and formation of a monoacetate **7b**, was confirmed by synthesis. Chol-5-ene-3β,24-diol (**7c**) was selectively converted to the 24-mono-*p*-toluenesulfonate (**7d**) which was reduced by lithium aluminum hydride to the required 24-deoxy steroid chol-5-en-3β-ol, identical with the suspected sample obtained from air-aged cholesterol.

The structure of 3β-hydroxycholest-5-ene 25-hydroperoxide (**3a**), that sterol hydroperoxide isolated from air-aged cholesterol in best yield (0.1–1%), was established by elemental analysis, formation of a diacetate **3b**,[10] reduction by sodium borohydride to the known cholest-5-ene-3β,25-diol (**4a**), positive peroxide color tests, and infrared absorption showing two strong OH stretching bands at 3620 and 3560 cm^{-1} characteristic of the hydroxyl and hydroperoxyl groups, respectively. Proton spectra of the diacetate **3b** showing the C_{26}- and C_{27}-methyl protons as a six-proton singlet at 1.28 ppm, deshielded 0.41 ppm by the 25-peracetoxyl group, complete the proof.[11]

A second sterol hydroperoxide 3β-hydroxycholest-5-ene 20α-hydroperoxide (**5a**) was similarly identified, with formation of a diacetate **5b** and sodium borohydride reduction to the known cholest-5-ene-3β,20α-diol (**6a**), positive peroxide color tests, characteristic absorption at 3620 and 3560 cm^{-1}, and proton spectra supporting the assigned structure. The C_{18}- and C_{21}-methyl group proton signals in the diacetate **5b** were singlets deshielded 0.18 and 0.41 ppm, respectively, by the 20α-peracetoxyl group.[12]

Other, as yet unidentified, sterol hydroperoxides X_1, X_2, and X_3 have been isolated and partially characterized. The structure of X_1, isolated in poor yield (0.002%) and separated from **3a** only with difficulty, as

(1) (a) Paper VII of the series "Sterol Metabolism." Paper VI: L. L. Smith and R. E. Gouron, *Water Res.*, **3**, 141 (1969). (b) Presented in part at the 5th International Symposium on the Chemistry of Natural Products, London, July 8–13, 1968, Abstracts, p 360, and before the 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 8–13, 1968, ORGN 58. (c) Supported financially by a grant from the U. S. Public Health Service (HE-10160).

(2) (a) L. L. Smith, W. S. Matthews, J. C. Price, R. C. Bachmann, and B. Reynolds, *J. Chromatogr.*, **27**, 187 (1967); (b) J. E. van Lier and L. L. Smith, *Biochemistry*, **6**, 3269 (1967); (c) J. E. van Lier and L. L. Smith, *Anal. Biochem.*, **24**, 419 (1968).

(3) For a review of steroids formed from cholesterol by autoxidation see (a) S. Bergström, *Ark. Kemi, Mineral., Geol.*, **16A**, No. 10, 1 (1942); (b) S. Bergström and B. Samuelsson in "Autoxidation and Antioxidants," Vol. 1, W. O. Lundberg, Ed., Interscience Publishers, New York, N. Y., 1961, pp 233–248; (c) L. F. Fieser and M. Fieser "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp 233–237; (d) P. Bladon in "Cholesterol, Chemistry, Biochemistry, and Pathology," R. P. Cook, Ed., Academic Press, Inc., New York, N. Y., 1958, pp 76–77.

(4) (a) G. O. Schenck, K. Gollnick, and O.-A. Neumüller, *Justus Liebigs Ann. Chem.*, **603**, 46 (1957); (b) G. O. Schenck and O.-A. Neumüller, *ibid.*, **618**, 194 (1958); (c) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **83**, 1498 (1961); (d) E. Caspi, J. B. Greig, P. J. Raum, and K. R. Varma, *Tetrahedron Lett.*, 3829 (1968).

(5) (a) G. O. Schenck, O.-A. Neumüller, and W. Eisfeld, *Angew. Chem.*, **70**, 595 (1958); (b) G. O. Schenck, O.-A. Neumüller, and W. Eisfeld, *Justus Liebigs Ann. Chem.*, **618**, 202 (1958); (c) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 471 (1959).

(6) (a) J. A. Fioriti and R. J. Sims, *J. Amer. Oil Chem. Soc.*, **44**, 221 (1967); (b) F. Neuwald and K.-E. Fetting, *Pharm. Ztg.*, **108**, 1490 (1963); (c) C. Horvath, *J. Chromatogr.*, **22**, 52 (1966).

(7) Sterol hydroperoxides have been demonstrated chromatographically in irradiated eggs[8a] and in formalinized human brain[8b] as artifacts. A stigmasterol hydroperoxide has been isolated from chestnut leaves under conditions which suggest that sterol hydroperoxides may occur in nature.[8c]

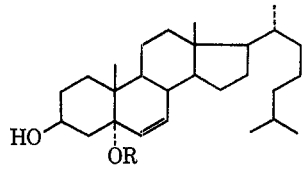
(8) (a) L. Acker and H. Greve, *Fette, Seifen, Anstrichm.*, **65**, 1009 (1963); (b) J. E. van Lier and L. L. Smith, *Tex. Rep. Biol. Med.*, **27**, 167 (1969); (c) F. G. Fischer and H. Mägerlin, *Justus Liebigs Ann. Chem.*, **636**, 88 (1960).

(9) (a) L. F. Fieser, W.-Y. Huang, and B. K. Bhattacharyya, *J. Org. Chem.*, **22**, 1380 (1957); (b) A. L. J. Beckwith, *Proc. Chem. Soc.*, 194 (1958).

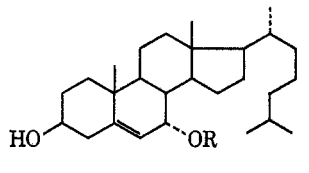
(10) Tertiary hydroperoxides are readily acetylated in distinction to tertiary alcohols; *cf.* E. L. Shapiro, T. Legatt, and E. P. Oliveto, *Tetrahedron Lett.*, 663 (1964).

(11) Compare deshielding of 0.56 ppm in **4b** by the 25-acetoxyl group which also reduces the normal doublet character of the C_{26}- and C_{27}-methyl proton signal in cholesterol to a singlet.

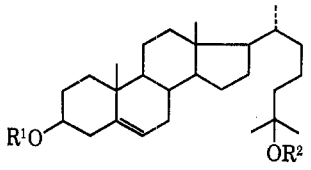
(12) Compare deshielding of 0.13 and 0.30 ppm, respectively, by the 20α-hydroxyl group in cholest 5-ene-3β,20α-diol; *cf.* A. Mijares, D. I. Cargill, J. A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).



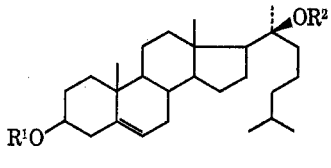
1a, R = OH
b, R = H



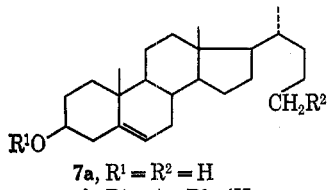
2a, R = OH
b, R = H



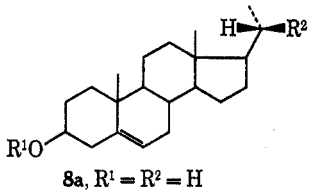
3a, $R^1 = H$; $R^2 = OH$
b, $R^1 = Ac$; $R^2 = OAc$
4a, $R^1 = R^2 = H$
b, $R^1 = R^2 = Ac$
c, $R^1 = Ac$; $R^2 = H$



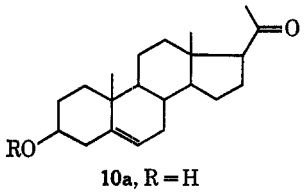
5a, $R^1 = H$; $R^2 = OH$
b, $R^1 = Ac$; $R^2 = OAc$
6a, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$



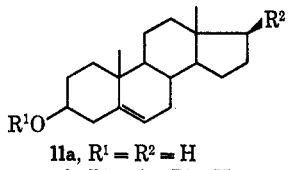
7a, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$
c, $R^1 = H$; $R^2 = OH$
d, $R^1 = H$; $R^2 = OTs$



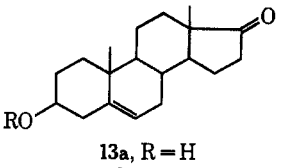
8a, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$
9a, $R^1 = H$; $R^2 = OH$
b, $R^1 = Ac$; $R^2 = OAc$



10a, R = H
b, R = Ac



11a, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$
12a, $R^1 = H$; $R^2 = OH$
b, $R^1 = Ac$; $R^2 = OAc$



13a, R = H
b, R = Ac

a cholesterol hydroperoxide was supported by high resolution mass measurement, positive peroxide color tests, strong absorption at 3620 and 3560 cm^{-1}, and sodium borohydride reduction to a new sterol diol not as yet identified. However, this sterol diol is not identical with the recently prepared cholest-5-ene-3β,17α-diol[13] or with 20-isocholest-5-ene-3β,20β-diol,[12] thus ruling out prospective 17α- or 20-iso-20β-hydroperoxide structures for X_1. Amounts of the other sterol hydroperoxides X_2 and X_3 obtained were inadequate for further chemical studies.

The presently observed autoxidation of cholesterol in the side chain is in clear distinction to previously described photoinduced singlet oxygen oxidation of cholesterol in the A/B ring system. Formation of the tertiary hydroperoxides **5a** and **3a** suggests that the autoxidative process involved is that of biradical oxygen attack at the preferred tertiary 20α- and 25-carbon atoms. In the same manner postulated for the decomposition of the 7α-hydroperoxide of cholesterol to give 7α- and 7β-hydroxy- and 7-oxo derivatives,[3d] so the decomposition of the 20α- and 25-hydroperoxides might be predicted to yield related tertiary alcohol and degraded ketone derivatives.

The 3β,25-diol **4a** already recognized as an autoxidation product of cholesterol[9] must be derived from the 25-hydroperoxide **3a**. Homolysis of the peroxide oxygen–oxygen bond and combination of the 25-alkoxy radical thereby produced with a hydrogen radical would afford the 3β,25-diol. A similar argument may be advanced to account for the presence of the 3β,20α-diol **6a** in air-aged cholesterol, by homolysis of the 20α-hydroperoxide oxygen–oxygen bond and combination with a hydrogen radical.

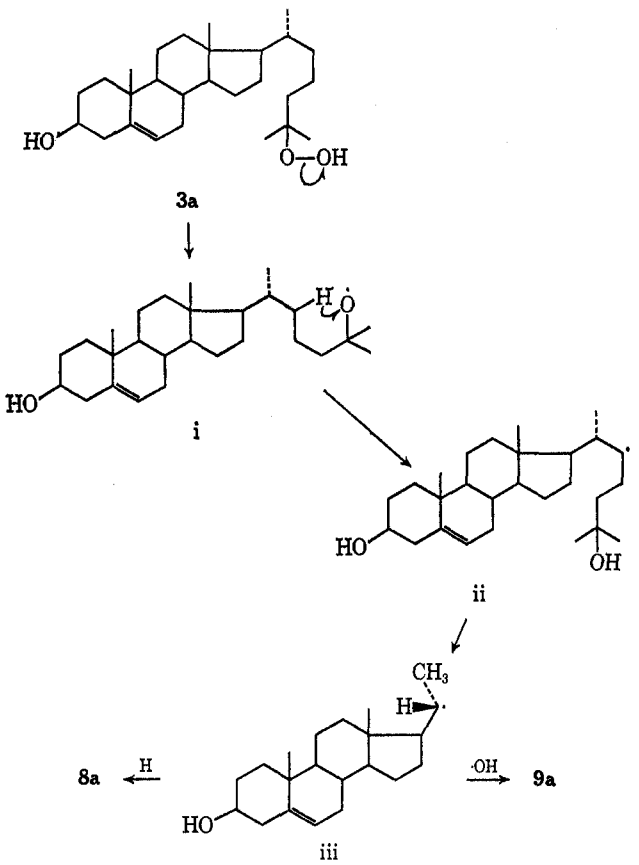
The degraded steroids **7a**, **8a**, **9a**, **10a**, **11a**, and **12a** may be derived from the 20α- and 25-hydroperoxides **5a** and **3a** by characteristic bond cleavage reactions involving radical intermediates. Thus, the 25-alkoxy radical obtained from the 25-hydroperoxide **3a** by homolysis, on β scission of the C_{25}–C_{26} bond, might yield 3β-hydroxy-27-norcholest-5-en-25-one, a product not detected in air-aged cholesterol in our studies despite a search for it. However, β scission of the C_{24}–C_{25} bond leading to a cholene 24 radical could then result by combination with a hydrogen radical in the formation of chol-5-en-3β-ol, which product was indeed isolated from air-aged cholesterol.

In the case of the 20α-hydroperoxide **5a**, β scission of the C_{20}–C_{22} bond in the 20α-alkoxy radical derived from **5a** by homolysis should result in formation of the 20 ketone **10a**, which product was isolated in our experiments. The presence of the 17 ketone **13a** in air-aged cholesterol may be accounted for by a similar sequence of radical reactions on a postulated cholesterol 17α-hydroperoxide not isolated in our present work.[14]

Scission of the C_{17}–C_{20} bond of the 20α-alkoxy radical derived from the 20α-hydroperoxide **5a** would give a 17 radical which on combination with a hydrogen radical would lead to androst-5-en-3β-ol, and with a hydroxyl radical would give the 3β,17β-diol **12a**. Both androstane derivatives were found in air-aged cholesterol. The similarly related pregnanes **8a** and **9a** may be viewed as originating from the 25-alkoxy radical initially formed from the 25-hydroperoxide **3a**, with a 1,5 migration of a 22 hydrogen *via* a cyclic transition state to yield a 25-hydroxy 22 radical which undergoes scission of the C_{20}–C_{22} bond to yield a pregnane 20 radical and the fragment 2-methylpent-4-en-2-ol. The 20 radical would afford pregn-5-en-3β-ol on combination with a hydrogen radical, the 3β,20α-diol **9a** on combination with a hydroxyl radical. Notably, no pregn-5-ene-3β,20β-diol was detected in our search of air-aged cholesterol. Accordingly, the 20-radical obtained on scission of the C_{20}–C_{22} bond retains configuration long enough for stereospecific recombination with hydroxyl radicals to give **9a**. In support of this formulation we have detected by gas chromatography and mass spectrometry the postulated fragment 2-

(13) N. K. Chaudhuri, R. Nicholson, and M. Gut, Abstracts of Papers, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 7–12, 1969.

(14) Cooccurrence of pregnane 17α-hydroperoxides and related 17 ketones derived therefrom has been noted; *cf.* W. P. Schneider and D. A. Ayer, Proceedings of the 2nd International Congress on Steroid Hormones. Milan, May 23–28, 1966, Excerpta Medica Foundation, Amsterdam, 1967, pp 254–260.



methylpent-4-en-2-ol in odorous material condensed from air-aged cholesterol.

The presence of androstane, pregnane, and cholane derivatives in air-aged cholesterol has not been noted heretofore. Examination of commercial cholesterol of recent manufacture or of purified cholesterol for these derivatives was without success. The controlled production of the hydroperoxides **3a** and **5a** in high-purity cholesterol heated in air can be observed chromatographically, and the concomitant formation of the degraded steroids may also be observed in such samples. We regard these observations as strong evidence that the degraded androstane, pregnane, and cholane derivatives isolated from air-aged cholesterol be derived from the 20α- and 25-hydroperoxides **5a** and **3a** formed in turn by biradical attack of triplet oxygen on cholesterol in the solid state.

Experimental Section[15]

Cholesterol Samples.—Initial isolation work was done with a lot of cholesterol distributed by Pfanstiehl Laboratories, Waukegan, Ill. (lot 1669, mp 147–148° at time of manufacture in 1947 by Wilson Laboratories, Chicago, Ill.), obtained as 10-g samples in unopened original brown glass vials sealed with cork and wax at manufacture, thus of an authenticated age of 20 years. Other commercial lots were used for additional isolation work to secure larger amounts of products. Highly purified cholesterol heated as a thin layer in an oven in the dark at 100° for 7 days afforded accelerated formation of the sterol hydroperoxides and the characteristic degradation products. The hydroperoxides **3a** and **5a** and associated degradation products could be detected in such pure cholesterol after 2 days of heating. At this point thin layer chromatograms of air-heated cholesterol resembled in detail the chromatograms of naturally air-aged cholesterol.[17] Prolonged heating led to increased difficulties in fractionation and to lower yields of the sterol hydroperoxides. The best hydroperoxide yields were obtained after heating pure cholesterol for 5–7 days with recrystallization of the oxidized batch from methanol and recovery of the oxidation products in the methanol mother liquor. The pure cholesterol was again subjected to heating for 5–7 days and recrystallization from methanol. The methanol mother liquors from five such 5–7-day heating periods were combined for fractionation. Yields of the hydroperoxides **3a** and **5a** varied in these heating experiments between 0.001 and 0.1%, with the 25-hydroperoxide **3a** predominating over **5a** and other hydroperoxides by a 10–100-fold factor.

Fractionation Procedure.—Batches of air-aged cholesterol (10–1000 g) were repeatedly recrystallized from methanol so as to give cholesterol and methanol mother liquors containing the autoxidation products. Exposure to light and air was minimized during all phases of fractionation, and thin layer chromatographic monitoring at each step suggested that the composition of autoxidation products was not altered appreciably. The autoxidation products were partitioned between methanol–hexane to remove apolar material (19% by weight, containing cholesta-3,5-dien-7-one) and diethyl ether–0.1 *N* sodium hydroxide solution to remove acidic material (16% by weight). The thoroughly washed and dried ether solution was evaporated under vacuum to yield a mobile oil enriched in autoxidation products of our interest.

Chromatography on silica gel using benzene–ethyl acetate gave select fractions containing **1a**, **3a**, **5a**, X_1, **4a**, **6a**, **7a**, **8a**, **9a**, **10a**, **11a**, **12a**, and **13a**, free from polar sterols (**2b**, *etc.*) but still containing cholesterol. Fractional crystallization from benzene

(15) Melting points were taken on a calibrated Kofler block under microscopic magnification. Optical rotations were obtained on 0.5–1% solutions in chloroform. Infrared absorption spectra were recorded over the range of 400–4000 cm^{-1} with a Perkin-Elmer Model 337 spectrophotometer equipped with a beam condenser, using 1.5-mm-diameter potassium bromide disks or as 0.003 *M* solutions in carbon tetrachloride (1-mm path). Proton spectra were recorded on 15% solutions in deuteriochloroform using a Varian Instruments Model A-60A spectrometer. Chemical shifts were recorded in parts per million downfield from an internal reference of tetramethylsilane. High-resolution mass spectra were obtained on a CEC Model 21-110B mass spectrometer, medium-resolution mass spectra on an LKB-9000 mass spectrometer. Elemental analyses were performed by the Huffman Laboratories, Wheatridge, Colo.

Ascending thin layer chromatography was conducted with benzene–ethyl acetate (3:2) on silica gel HF_{254} chromatoplates using techniques previously described.[2a] Visualization of steroids was by means of 50% aqueous sulfuric acid spray. Gas chromatography was conducted on 3% SE-30 and 3% QF-1 columns by means already described.[2b,c] Preparative gas chromatography on 3% QF-1 columns[16a] and column chromatography on Sephadex LH-20[16b] (Pharmacia Fine Chemicals Inc., Uppsala) were conducted as described elsewhere in detail. Chromatographic behavior for steroids is given in the order: thin layer mobility in benzene–ethyl acetate (3:2) as R_C values with cholesterol as unit mobility; color response to 50% sulfuric acid, in parentheses; relative retention times (r_T) on 3% QF-1 and on 3% SE-30, with cholesterol serving as unity.

(16) (a) J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **36**, 7 (1968); (b) J. E. van Lier and L. L. Smith, *ibid.*, **41**, 37 (1969).

(17) Very complex thin layer chromatograms result.[2a,6c] Sterol hydroperoxides are not resolved as a group on such chromatograms but are recognized among other steroid products by selective response to both potassium iodide–starch and ferrous thiocyanate color tests for peroxides.[18] Color responses to 50% sulfuric acid were not sufficiently distinctive for confident recognition of the hydroperoxides in mixtures. As an adjunct means of recognition of sterol hydroperoxides on thin layer chromatograms a 2–10-μg sample of mixed sterols was spotted as usual, and 5 μl of a freshly prepared 1% solution of sodium borohydride in methanol was carefully spotted directly over the sample spot. After the methanol evaporated the chromatoplate was irrigated as usual with benzene–ethyl acetate (3:2) and visualized with 50% sulfuric acid. Careful comparison of the borohydride reduced chromatograms *vs.* the same sample not reduced before irrigation permitted recognition of a change in mobility and of color-test response for the sterol hydroperoxides, whose mobilities after reduction matched identically those of the known hydroxylated cholesterol reference samples. Although the sterol hydroperoxides **3a**, **5a**, X_1, and X_2 are not well resolved from one another on adsorption mode thin layer chromatograms, the reduced derivatives **4a** and **6a** and the alcohol derived from X_1 are readily resolved thereby, thus permitting direct thin layer chromatographic analysis of the composition of a given mixture of sterol hydroperoxides containing **3a**, **5a**, and X_1. Thin layer chromatographic mobility and color response to sulfuric acid characterize the hydroperoxides and their respective borohydride reduction products as follows: **1a**, 0.59 (green-blue); **2a**, 0.64 (green-blue); **3a**, 0.84 (brown-red); **5a**, 0.93 (gray-brown); X_1, 0.95 (gray-brown); X_2, 0.82 (gray-brown); X_3, 0.62 (brown-red); **1b**, 0.35 (sky blue); **2b**, 0.22 (sky blue); cholest-5-ene-3β,7β-diol, 0.27 (sky blue); **4a**, 0.57 (purple-blue); **6a**, 0.88 (green-blue); X_1 alcohol, 0.77 (red-brown); X_2 alcohol, 0.74 (green-blue); X_3 alcohol, 0.24 (gray-brown).

(18) D. Waldi in "Thin-Layer Chromatography, A Laboratory Handbook," E. Stahl, Ed., Springer-Verlag, Berlin, 1965, pp 483–502.

removed cholesterol and afforded 6 g of **4a** (from 1 kg of air-aged cholesterol). Rechromatography of the benzene mother liquors of silica gel with benzene–ethyl acetate gave a more mobile fraction containing the steroids of our interest and a more polar fraction containing the 5α-hydroperoxide **1a**, **4a**, **9a**, and **12a**.

3β-Hydroxy-5α-cholest-6-ene 5-Hydroperoxide (1a) and 3β-Hydroxycholest-5-ene 7α-Hydroperoxide (2a).—The more polar sterol fraction was shown to contain **1a** by thin layer chromatography, both by direct observation and by the borohydride reduction procedure. Gas chromatography showed cracking patterns characteristic of authentic **1a** (peaks at r_T 0.53 and 0.47 on 3% QF-1). The fraction was chromatographed on Sephadex LH-20 in neat methylene chloride, but the hydroperoxide recovered was **2a**, recrystallized from hexane–diethyl ether, mp 154–158° dec (lit. mp 154° dec,[5a] 154–155° dec,[5c] 154–156.5° dec,[5b] 154–156°[4d]); $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050 cm^{-1}, different from spectra of **1a**. Gas chromatography gave a decomposition pattern characteristic of authentic **2a** (r_T 0.47 and 4.70, due to 3β-hydroxycholest-5-en-7-one). Authentic **1a** [mp 149–151° dec (lit. mp 142° dec,[4a] 148–149° dec,[4b] 145–148° dec,[5b] 149.5–150.5°,[4c] 147–149°[4d]); $\tilde{\nu}_{max}^{KBr}$ 3400, 3200, 1620, 1040, 1020 cm^{-1}] subjected to similar chromatography was completely isomerized to **2a**, mp 154–158°.

Chromatography on Sephadex LH-20.—The more mobile fraction containing autoxidation products of our interest was chromatographed with methylene chloride–acetone (9:1) on Sephadex LH-20,[16b] a key procedure in the resolution of the hydroperoxides. Group fractions obtained in order were (A) cholesterol, monohydroxy steroids (**7a**, **8a**, **11a**), and hydroxy ketones (**10a**, **13a**), together with the 3β,20α-diol **6a**; (B) the polar diol fraction (**4a**, **9a**, **12a**); and (C) the substantially retarded hydroperoxide fraction (**3a**, **5a**, X_1, X_2, X_3).

After fractional crystallization of more cholesterol from the A fraction the material was subjected to Girard T separation.[19] From the ketonic fraction after preparative thin layer chromatography using benzene–ethyl acetate (3:2) there was isolated the two ketones, **10a** and **13a**.

3β-Hydroxypregn-5-en-20-one (10a).—Material from the more mobile band from the preparative thin layer chromatogram was eluted with methanol–chloroform (1:2) and recrystallized from hexane–diethyl ether to give **10a**, 12 mg, mp 191–192°, not depressed on admixture with an authentic sample (lit.[20] mp 188–194°); $\tilde{\nu}_{max}^{KBr}$ 3420, 1690, 1620, 1060 cm^{-1}; R_C 0.74 (red-orange); r_T 0.97, 0.33; identical in these properties with authentic **10a**.

Acetylation with acetic anhydride–pyridine in the usual manner gave the monoacetate **10b**, mp 145.0–145.5°, not depressed on admixture with authentic **10b** (lit.[20] mp 145–150°); $\tilde{\nu}_{max}^{KBr}$ 1720, 1690, 1620, 1240, 1030 cm^{-1}; R_C 1.42 (red-orange); r_T 1.50, 0.49; identical in all respects with authentic **10b**.

3β-Hydroxyandrost-5-en-17-one (13a).—The more polar component from the Girard ketonic fraction eluted from the thin layer chromatoplate with methanol–chloroform (1:2) was recovered by preparative gas chromatography. Only a very small amount of this sample could be obtained, mp 125° (lit.[20] mp 145–150°), and further purification to a satisfactory melting point could not be achieved. Authentic **13a** subjected to preparative gas chromatography on 3% QF-1 in the same manner likewise exhibited the characteristic, depressed melting point. Identity of the sample was assured on other criteria: $\tilde{\nu}_{max}^{KBr}$ 3420, 1740, 1730, 1620, 1060 cm^{-1}; R_C 0.65 (purple red); r_T 0.75, 0.21; identical in these properties with authentic **13a**.

Acetylation of the material with acetic anhydride–pyridine in the usual manner gave a product recovered by gas chromatography and crystallized from chloroform, identified as the monoacetate **13b**, mp 165–170° (lit.[20] mp 168–172°); $\tilde{\nu}_{max}^{KBr}$ 1730, 1740, 1745, 1620, 1240, 1020 cm^{-1}; R_C 1.26 (purple-red); r_T 1.16, 0.30; identical in these properties with authentic **13b**.

The Girard nonketonic fraction was chromatographed on a 2-mm thick silica gel PF_{254} chromatoplate to resolve the alcohols **7a**, **8a**, and **11a** with mobility of cholesterol from the diol **6a**. The mixed alcohols were eluted together and resolved by gas chromatography on 3% QF-1, giving chromatographically pure preparations but with melting points some 5–10° below those of authentic samples.

Androst-5-en-3β-ol (11a).—The first-eluted alcohol **11a** was recromatographed on 3% QF-1 and identified by spectral and chromatographic data: $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050 cm^{-1}; R_C 0.98 (magenta); r_T 0.21, 0.10; identical in these properties with authentic **11a**, mp 130–134° (lit.[20] mp 131°), prepared by lithium aluminum hydride reduction of 3β-acetoxyandrost-5-en-17β-ol *p*-toluenesulfonate.

Acetylation of **11a** with acetic anhydride–pyridine in the usual fashion with purification by preparative gas chromatography, gave the monoacetate, crystallized from chloroform to give pure **11b**, mp 89–92°, not depressed on admixture with authentic **11b**, mp 90–92° (lit.[20] mp 91–93°); $\tilde{\nu}_{max}^{KBr}$ 1730, 1620, 1240, 1030 cm^{-1}; R_C 1.55 (magenta); r_T 0.31, 0.16; identical in these properties with authentic **11b**.

Pregn-5-en-3β-ol (8a).—The middle component resolved on 3% QF-1 gas chromatographic columns of mixtures of **11a**, **8a**, and **7a** was the alcohol **8a**, crystallized from diethyl ether; $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050 cm^{-1}; R_C 0.96 (magenta); r_T 0.31, 0.21; identical in these properties with authentic **8a**, mp 135–136° (lit.[20] mp 134–136°), prepared by lithium aluminum hydride reduction of the 20α-*p*-toluenesulfonate 3β-acetate diester of **9a**.

Acetylation of **8a** isolated from air-aged cholesterol with acetic anhydride–pyridine in the usual manner, followed by purification by preparative gas chromatography and recrystallization from chloroform, gave the 3β-monoacetate **8b**, mp 147–148°, not depressed on admixture with authentic **8b**, mp 148–150° (lit.[20] mp 147–150°); $\tilde{\nu}_{max}^{KBr}$ 1730, 1620, 1240, 1030 cm^{-1}; R_C 1.56 (magenta); r_T 0.52, 0.29; identical in these properties with authentic **8b**.

Chol-5-en-3β-ol (7a). (A) From Air-Aged Cholesterol.—The third component effluxed from the preparative gas chromatogram of the mixture of **11a**, **8a**, and **7a** was **7a**, crystallized from diethyl ether, mp 120–125°, depressed from gas chromatography; $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1060, 1040 cm^{-1}; R_C 1.00 (magenta); r_T 0.62, 0.47; identical in these properties with authentic **7a** prepared under (**B**) below.

(B) From 3β-Acetoxychol-5-enic Acid.—A solution of 2 g of 3β-acetoxychol-5-enic acid in diethyl ether was refluxed overnight with excess lithium aluminum hydride. The mixture was poured onto ice, acidified with 50% hydrochloric acid, and extracted twice with 100-ml portions of diethyl ether. The pooled ether extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated under vacuum. Crystallization of the residue from ethyl acetate gave 1.2 g of the 3β,24-diol **7c**, mp 194–196° (lit.[20] mp 193–195°). A solution of 10 g of **7c** in 25 ml of dry pyridine at 0° was treated with 5 g of *p*-toluenesulfonyl chloride. The progress of the reaction was monitored by thin layer chromatography and the reaction was terminated by pouring into ice when about half complete. The precipitate obtained was filtered, washed with water, dried under vacuum, and redissolved in diethyl ether. Without separating the 3β,24-diol **7c** and the 24-mono-*p*-toluenesulfonate **7d**, the reaction mixture was refluxed overnight with excess lithium aluminum hydride and worked up in the manner described above, yielding a mixture of **7a** and **7c**. After chromatography on silica gel using hexane–diethyl ether (7:3), there was obtained 160 mg of pure **7a**, mp 127–130°; $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1060, 1040 cm^{-1}; R_C 1.00 (magenta); r_T 0.62, 0.47.

Anal. Calcd for $C_{24}H_{40}O$ (344.56): C, 83.66; H, 11.70. Found: C, 83.60; H, 11.83.

Chol-5-en-3β-ol 3β-Acetate (7b). (A) From Air-Aged Cholesterol.—The sample of **7a** recovered from air-aged cholesterol was acetylated with acetic anhydride–pyridine in the usual manner to give the monoacetate **7b**, recrystallized from chloroform, mp 125–128°, not depressed on admixture with authentic **7b**; $\tilde{\nu}_{max}^{KBr}$ 1725, 1620, 1250, 1040 cm^{-1}; R_C 1.57 (magenta); r_T 1.16, 0.70; identical in all respects with authentic **7b**.

(B) From Synthesis.—Acetylation of **7a** derived by synthesis with acetic anhydride in the usual fashion gave an acetylated product which after chromatography on silica gel was recrystallized from methanol, yielding 40 mg of pure **7b**, mp 127–129°; $\tilde{\nu}_{max}^{KBr}$ 1725, 1620, 1250, 1040 cm^{-1}; R_C 1.57 (magenta); r_T 1.16, 0.70.

Anal. Calcd for $C_{26}H_{42}O_2$ (386.60): C, 80.77; H, 10.95. Found: C, 80.86; H, 10.78.

Cholest-5-ene-3β,20α-diol (6a). (A) From Air-Aged Cholesterol.—The second, less mobile band on the preparative thin layer chromatogram of the nonketone fraction from which **11a**, **8a**, and **7a** had been isolated gave the 3β,20α-diol **6a** on rechromatography, crystallized from hexane–ethyl acetate; $\tilde{\nu}_{max}^{KBr}$ 3400,

(19) J. J. Schneider, *J. Biol. Chem.*, **183**, 365 (1950); **194**, 337 (1952).

(20) Melting points of well-known steroids are taken from the compendium, J. Jacques, H. Kagan, G. Ourisson, "Selected Constants, Optical Rotatory Power. Ia. Steroids," Vol. 14 of "Tables of Constants and Numerical Data," S. Allard, Ed., Pergamon Press, Oxford, 1965.

1620, 1060 cm^{-1}; R_C 0.93 (green-blue); r_T 2.09, 1.56; identical in these properties with authentic **6a**. A sample of the 20α-hydroperoxide **5a** reduced in methanol with sodium borohydride also gave the 3β,20α-diol **6a**, identified by spectral and chromatographic properties with authentic **6a**.

Acetylation of **6a** derived from air-aged cholesterol and of **6a** derived by borohydride reduction of **5a** with acetic anhydride–pyridine in the usual manner gave the 3β-monoacetate **6b**, purified by gas chromatography, mp 153–157°, not depressed on admixture with authentic **6b** (lit.[20] mp 156–157°); $\tilde{\nu}_{max}^{KBr}$ 3570, 1720, 1620, 1260, 1030 cm^{-1}; R_C 1.40 (green-blue); r_T 3.00, 2.21; identical in all respects with authentic **6b**.

Cholest-5-ene-3β,25-diol (4a).—The diol fraction (B) from the Sephadex LH-20 column contained **4a**, **9a**, and **12a** together with unidentified components. Fractional crystallization of the mixture from ethyl acetate gave **4a**, mp 172–176°, not depressed on admixture with authentic **4a** (lit.[20] mp 172–183°); $\tilde{\nu}_{max}^{KBr}$ 3330, 1620, 1060 cm^{-1}; R_C 0.57 (purple-blue); r_T 2.38, 1.64; identical in these properties with authentic **4a**. The total yield of **4a** from this and other fractions was 15 g/kg of air-aged cholesterol.

Reduction of **3a** (50 mg) in methanol by sodium borohydride gave **4a** (28 mg), mp 180.5–181.5°, not depressed on admixture with authentic **4a**, identical by spectral and chromatographic comparison with authentic **4a**.

Acetylation of 10 mg of **4a** from air-aged cholesterol with acetic anhydride–pyridine in the usual manner gave the 3β-monoacetate **4c**, mp 139–140°, not depressed on admixture with authentic **4c** (lit.[20] mp 138–142°); $\tilde{\nu}_{max}^{KBr}$ 3450, 1730, 1620, 1250, 1030 cm^{-1}; R_C 1.11 (purple-red); r_T 3.82, 2.32; identical in all respects with authentic **4c**. A sample of **4c**, mp 139–140°, derived from **3a**, was similarly identified by spectral and chromatographic data.

Pregn-5-ene-3β,20α-diol (9a).—The mother liquor remaining after crystallization of **4a** was chromatographed on Sephadex LH-20 and the fractions containing **9a** still contaminated with **4a** were resolved on thin layer chromatoplates using benzene–ethyl acetate (3:2), thus yielding **9a**, 8 mg, crystallized from ethyl acetate, mp 183–184°, not depressed on admixture with authentic **9a** (lit.[20] mp 177–184°); $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050 cm^{-1}; R_C 0.41 (red-purple); r_T 0.72, 0.38; identical in all respects with authentic **9a**.

Acetylation of **9a** with acetic anhydride–pyridine in the usual manner gave the diacetate **9b**, purified by additional thin layer and gas chromatography, mp 143–144°, not depressed on admixture with an authentic sample of **9b** (lit.[20] mp 142–147°); $\tilde{\nu}_{max}^{KBr}$ 1730, 1620, 1250, 1030 cm^{-1}; R_C 1.44 (red-purple); r_T 1.74, 0.74; identical in all respects with authentic **9b**.

Androst-5-ene-3β,17β-diol (12a).—The 3β,17β-diol **12a** was eluted after **9a** from the Sephadex LH-20 column of the diol fraction (B). After thin layer chromatography and recrystallization from ethyl acetate and from hexane–ethyl acetate the pure sample was obtained, mp 178–179°, not depressed on admixture with authentic **12a** (lit.[20] mp 174–184); $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050 cm^{-1}; R_C 0.41 (red-purple); r_T 0.44, 0.22; identical in all respects with authentic **12a**.

Acetylation of **12a** with acetic anhydride in the usual manner gave the 3β,17β-diacetate **12b**, mp 158.0–159.5°, not depressed on admixture with authentic **12b** (lit.[20] mp 156–158°); $\tilde{\nu}_{max}^{KBr}$ 1730, 1620, 1250, 1040 cm^{-1}; R_C 1.32 (red-purple); r_T 1.13, 0.45; identical in all respects with authentic **12b**.

3β-Hydroxycholest-5-ene 25-Hydroperoxide (3a).—The retarded hydroperoxide fraction (C) from the key Sephadex LH-20 chromatographic column contained **3a**, **5a**, X_1, X_2, and X_3. Crystallization of the hydroperoxide fraction from ethyl acetate afforded 1.2 g of **3a** (from 1 kg of aged cholesterol), mp 157–158°; [α]D −39° (*c* 0.94); $\tilde{\nu}_{max}^{KBr}$ 3400, 3270 (shoulder), 3180 (shoulder), 1630, 1050 cm^{-1}; $\tilde{\nu}_{max}^{CCl_4}$ 3620 (hydroxyl), 3560 (hydroperoxyl) cm^{-1}; R_C 0.84 (brown-red).

Anal. Calcd for $C_{27}H_{46}O_3$ (418.63); C, 77.46; H, 11.07. Found: C, 77.36; H, 11.22.

3β-Acetoxycholest-5-ene 25-Hydroperoxide 25-Acetate (3b).—A solution of 80 mg of **3a** in 3 ml of dry pyridine–acetic anhydride (2:1) was held overnight at room temperature. The solution was poured into ice water, and the mixture was extracted several times with chloroform. The chloroform solution was evaporated under vacuum and the residue was crystallized from methanol, yielding 76 mg of **3b**, mp 94.2–96.3°; [α]D −40° (*c* 0.936); $\tilde{\nu}_{max}^{KBr}$ 1770, 1720, 1630, 1250, 1190, 1040 cm^{-1}; R_C 1.52 (brown-red). A characteristic decomposition pattern was obtained on gas chromatography of **3b**.

Anal. Calcd for $C_{31}H_{50}O_5$ (502.73): C, 74.06; H, 10.03. Found: C, 74.11; H, 10.29.

Proton spectra of **3b** included signals at (δ in ppm) 0.70 (3 H, C_{18} protons), 0.93 (d, J = 5 Hz, 3 H, C_{21} protons), 1.05 (3 H, C_{19} protons), 1.28 (6 H, C_{26} and C_{27} protons), 2.05 (3 H, 3β-acetoxyl protons), 2.08 (3 H, 25-peracetoxyl protons), 4.63 (m, $W_{1/2}$ = 25 Hz, 1 H, 3α proton), 5.36 (d, J = 4 Hz, 1 H, C_6-vinyl proton). For comparison cholest-5-ene-3β,25-diol 3β,25-diacetate (**4b**): 0.69 (3 H, C_{18} protons), 0.95 (d, J = 5 Hz, 3 H, C_{21} protons), 1.02 (3 H, C_{19} protons), 1.43 (6 H, C_{26} and C_{27} protons), 1.95 (3 H, 25-acetoxyl protons), 2.02 (3 H, 3β-acetoxyl protons), 4.63 (m, $W_{1/2}$ = 25 Hz, 1 H, 3α proton), 5.35 (d, J = 4 Hz, 1 H, C_6-vinyl proton); and cholesterol acetate: 0.70 (3 H, C_{18} protons), 0.87 (d, J = 7 Hz, 6 H, C_{26} and C_{27} protons), 0.91 (d, J = 6 Hz, C_{21} protons), 1.04 (3 H, C_{19} protons), 2.03 (3 H, 3β-acetoxyl protons), 4.60 (m, $W_{1/2}$ = 25 Hz, 1 H, 3α proton), 5.38 (d, J = 4 Hz, 1 H, C_6-vinyl proton).

3β-Hydroxycholest-5-ene 20α-Hydroperoxide (5a).—The mother liquor containing **5a**, X_1, X_2, and X_3 after isolation of **3a** was chromatographed on silica gel and then on Sephadex LH-20 using neat methylene chloride. From the appropriate fractions **5a** was recovered by crystallization (80 mg), and rechromatography of the mother liquor afforded 40 mg of **5a** (120 mg/kg of air-aged cholesterol), mp 146.0–149.5°; [α]D −66° (*c* 0.714); $\tilde{\nu}_{max}^{KBr}$ 3400, 1630, 1050 cm^{-1}; $\tilde{\nu}_{max}^{CCl_4}$ 3620, 3560 cm^{-1}; R_C 0.93 (gray-brown).

Anal. Calcd for $C_{27}H_{46}O_3$ (418.63): C, 77.46; H, 11.07. Found: C, 77.67; H, 10.99.

3β-Acetoxycholest-5-ene 20α-Hydroperoxide 20α-Acetate (5b).—Acetylation of **5a** with acetic anhydride–pyridine in the usual fashion gave the diacetate **5b**, chromatographed on silica gel and recrystallized from methanol, 30 mg, mp 75–80°; [α]D −0.3° (*c* 0.556); $\tilde{\nu}_{max}^{KBr}$ 1770, 1720, 1630, 1250, 1190, 1030 cm^{-1}; R_C 1.52 (gray-brown). A characteristic decomposition pattern was obtained on gas chromatography of **5b**.

Anal. Calcd for $C_{31}H_{50}O_5$ (502.71): C, 74.06; H, 0.03. Found: C, 73.80; H, 10.10.

Proton spectra (δ in ppm) of **5b** included 0.88 (3 H, C_{18} protons), 0.89 (d, J = 6 Hz, 6 H, C_{26} and C_{27} protons), 1.03 (3 H, C_{19} protons), 1.32 (3 H, C_{21} protons), 2.01 (3 H, 20α-peracetoxyl protons), 2.04 (3 H, 3β-acetoxyl protons), 4.60 (1 H, 3α proton), 5.38 (1 H, C_6-vinyl proton).

Unidentified Cholesterol Hydroperoxide (X_1).—After elution of the 20α-hydroperoxide **5a** from the second Sephadex LH-20 chromatographic column a small fraction containing X_1 contaminated with **3a** and **5a** was recovered. Continued elution of the column gave more **3a** (300 mg). Rechromatography of the mixed hydroperoxide fractions on Sephadex LH-20 and on silica gel, using a 0–5% linear gradient of ethyl acetate in benzene, gave fractions of X_1 free from **3a** and **5a**. After recrystallization of the hydroperoxide from benzene–ethyl acetate there was obtained 30 mg of pure X_1 free from **3a** and **5a**, mp 160–164°; $\tilde{\nu}_{max}^{KBr}$ 3400, 1630, 1050 cm^{-1}; $\tilde{\nu}_{max}^{CCl_4}$ 3620, 3560 cm^{-1}; R_C 0.95 (gray-brown); it gave a characteristic decomposition pattern on gas chromatography.

Anal. Calcd for $C_{27}H_{46}O_3$: M, 418.3446. Found: M, 418.3473.

Acetylation of 15 mg of X_1 with acetic anhydride–pyridine at room temperature overnight in the usual manner afforded a product which migrated as a single component on thin layer chromatograms (R_C 1.50) but which was resolved into two steryl acetate components on gas chromatography: X_1-a, 68%, r_T, 4.75, 2.43; X_1-b, 32%, r_T 4.28, 2.16 not worked on further.

A sample of mixed hydroperoxides enriched in X_1 was reduced at room temperature as a methanol solution with excess sodium borohydide for 15 min. The reduced product was purified by preparative thin layer chromatography, and the crude reduced product was crystallized from benzene, mp 176–179°; $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1050, 1020 cm^{-1}; R_C 0.77 (red-brown) r_T 2.18, 1.86.

2-Methylpent-4-en-2-ol.—A ten-year old 1-kg sample of air-aged cholesterol in a 5-l. flask was subjected to vacuum for 5 days. An odorous volatile fraction (2 ml/kg) collected in a cold (−50°) trap was shown to be a mixture of low-molecular-weight components by gas chromatography on a 500 ft × 0.03 in. diameter metal capillary column coated with SF-96 silicone oil, using helium as a carrier at 15 ml/min and column temperature 20°. Selected effluent peaks were analyzed in the LKB Model 9000 mass spectrometer, the total ion current being plotted to give an elution curve. That peak recognized as 2-methylpent-4-en-2-ol also contained 2-methylpentan-2-ol, as recognized by

additional gas chromatography on Poropak Q columns (at 230°). Mass spectral analysis of the mixed alcohol fraction gave ions consistent with the structures of the two components, thus ions at m/e 85 $(M - CH_3)^+$, 59 $(C_3H_7O)^+$, 43 $(C_3H_7)^+$, and 31 $(CH_3O)^+$ associated with 2-methylpent-4-en-2-ol and ions at 87 $(M - CH_3)^+$, 69 $(C_5H_9)^+$, 59 $(C_3H_7O)^+$, 45 $(C_2H_5O)^+$, 43 $(C_3H_7)^+$, and 31 $(CH_3O)^+$ associated with 2-methylpentan-2-ol.

Mass spectra of the reference alcohols (Aldrich Chemical Co. Inc., Milwaukee, Wis.) are given for comparison (with per cents in parentheses): 2-methylpent-4-en-2-ol m/e 85 (5), 83 (3), 59 (100), 55 (8), 43 (82), 41 (42), 39 (55), 31 (35), 29 (9), 27 (24); 2-methylpentan-2-ol 87 (21), 85 (3), 69 (7), 59 (94), 45 (37), 43 (100), 41 (30), 39 (28), 31 (20), 29 (15), 27 (38).

Registry No.—Cholesterol, 57-88-5; **3a**, 23652-97-3; **3b**, 23652-98-4; **4a**, 2140-46-7; **4c**, 10525-22-1; **5a**, 23653-01-2; **5b**, 23653-02-3; **6a**, 516-72-3; **6b**, 7484-20-0; **7a**, 5255-15-2; **7b**, 23653-06-7; **8a**, 2862-58-0; **8b**, 3090-79-7; **9a**, 901-56-4; **9b**, 1913-47-9; **10a**, 145-13-1; **10b**, 1778-02-5; **11a**, 1476-64-8; **11b**, 13067-44-2; **12a**, 521-17-5; **12b**, 2099-26-5; **13a**, 53-43-0; **13b**, 853-23-6.

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A Study of Amine-Catalyzed Epimerization of 2β-Methylcholestan-3-one[1]

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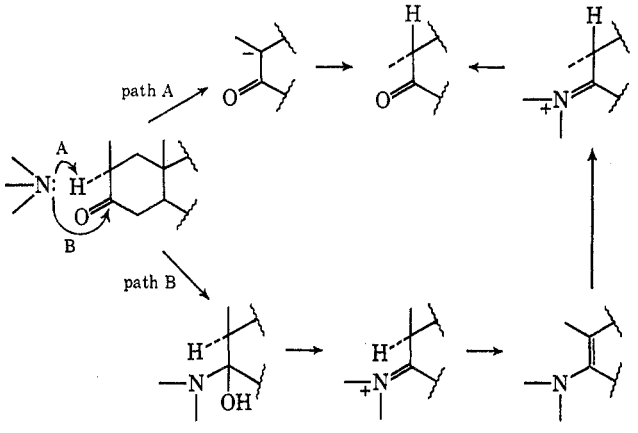
The epimerization of 2β-methylcholestan-3-one (1) to 2α-methylcholestan-3-one (2) in dioxane solution at 45° in the presence of an excess of various amines has been followed by optical rotation measurements. The results contrast with those previously found for the conversions **3** → **5** and **4** → **5**; piperidine was about as catalytically effective as pyrrolidine and hexamethylenimine, and the unhindered tertiary amine quinuclidine was only slightly less effective. These observations suggest that nucleophilic catalysis is less important in the conversion **1** → **2**, presumably because the 2β-methyl group inhibits enamine formation.

The primary question one seeks to answer in any investigation of the mechanism of an amine-catalyzed carbonyl compound reaction is whether the amine acts as a general base to remove a proton directly (as in path A below), or as a nucleophile to form an enamine which can react analogously to the enolate anion and then be reconverted to the carbonyl compound by hydrolysis (path B). Although the latter pathway was proposed many years ago,[2,3] systematic studies of this type of catalysis have appeared only within the last decade.[4-7] Most of these studies were presumably inspired by the increasing evidence of nucleophilic catalysis by amines in certain biochemical reactions.[8]

Much of this evidence comes from trapping substrate–enzyme imine intermediates by borohydride reduction.[9] However, these experiments do not prove that the imine is necessarily an intermediate and do not provide quantitative information about catalytic effectiveness. Kinetic investigations of model systems[2-7] designed to elucidate these matters by comparison of the catalytic rate constants for different amines have largely relied on relative inactivity of tertiary amines[3] and other deviations from the Brønsted catalysis law[6] as criteria for nucleophilic catalysis.



This paper describes our investigation of the epimerization of 2β-methylcholestan-3-one (1) to 2α-methylcholestan-3-one (2). This reaction was selected in order to provide a contrast to our previous study[10] of the aldol condensation–ketol dehydration sequence **3** → **4** → **5**, in which nucleophilic catalysis was inferred not only from the relative inefficiency of tertiary amines, but also from a comparison of the rates of reactions catalyzed by the three cyclic secondary amines pyrrolidine, piperidine, and hexamethylenimine. These amines are of similar base strength and steric bulk, and their kinetic basicity with respect to a given weak car-

(1) This research was presented at the Fifth Caribbean Chemical Symposium, University of the West Indies, Cave Hill, Barbados, Jan 1969.

(2) K. J. Pedersen, *J. Phys. Chem.* **38**, 559 (1934).

(3) F. H. Westheimer and H. Cohen, *J. Amer. Chem. Soc.*, **60**, 90 (1938); F. H. Westheimer, *Ann. N. Y. Acad. Sci.*, **39**, 401 (1940); F. H. Westheimer and W. A. Jones, *J. Amer. Chem. Soc.*, **63**, 3283 (1941).

(4) J. Hine, F. E. Rogers, and R. E. Notari, *ibid.*, **90**, 3279 (1968), and previous papers in this series.

(5) L. P. Koshechkina, E. A. Shilov, and A. A. Yasnikov, *Ukr. Khim. Zh.*, **35**, 55 (1969), and previous papers in this series. These extensive reports by Yasnikov and coworkers contain many arguable mechanistic suggestions. Discussion of these papers has been promised by Hine [J. Hine, B. C. Menon, J. H. Jensen, and J. Mulders, *J. Amer. Chem. Soc.*, **88**, 3367 (1966)].

(6) M. L. Bender and A. Williams, *ibid.*, **88**, 2502 (1966).

(7) G. E. Lienhard and T-C. Wang, *ibid.*, **90**, 3781 (1968).

(8) See W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 2, especially pp 116–146, for a review and specific references.

(9) For example: (a) S. Warren, B. Zerner, and F. H. Westheimer, *Biochemistry*, **5**, 817 (1966); (b) J. C. Speck, Jr., P. T. Rowley, and B. L. Horecker, *J. Amer. Chem. Soc.*, **85**, 1012 (1963), and B. L. Horecker, T. Cheng, E. Grazi, C. Y. Lai, P. Rowley, and O. Tchola, *Fed. Proc.*, **21**, 1023 (1962); (c) D. Portsmouth, A. C. Stoolmiller, and R. H. Abeles, *J. Biol. Chem.*, **242**, 2751 (1967).

(10) T. A. Spencer, H. S. Neel, T. W. Flechtner, and R. A. Zayle, *Tetrahedron Lett.*, 3889 (1965).