analyzed on a diisodecyl phthalate column, two components in about equal amounts with retention times identical with those of **15** and **16** were found. Nmr and ir spectra of a mixture of authentic **15** and **16**[24] in the same ratio agreed with the spectra obtained from the preparative gc sample. Compound **13** could not be isolated free of **14**, but the nmr of the mixture had the characteristic absorption of a 3,4-unsaturated ketone: nmr (CCl_4) δ 2.43 ($CCH_2C{=}O$), 2.78 ($CH{=}CHCH_2C{=}O$), 5.79 ($CH{=}CH$), the remaining CH_2 was obscured by **14**.

An analytical sample of the monotosylhydrazone of **12** was recrystallized from methanol, mp 208.3–209.0° dec.

Anal. Calcd for $C_{13}H_{16}N_2SO_3$: C, 55.69; H, 5.75; N, 9.99. Found: C, 55.75; H, 5.62; N, 10.25.

Registry No.—**1**, 126-81-8; **2**, 41189-09-7; **3**, 4694-17-1; **4**, 41189-10-0; **6**, 20500-49-6; **7**, 1121-18-2; **8**, 1193-55-1; **8** monotosylhydrazone, 41189-12-2; **11**, 930-68-7; **12**, 504-02-9; **12** monotosylhydrazone, 41189-13-3; **13**, 4096-34-8; tosylhydrazide, 1576-35-8; 3-methyl-2-cyclopenten-1-one, 2758-18-1; methyl iodide, 74-88-4.

(24) Compound **16** was synthesized from 2-carbethoxycyclopentanone: F. C. Case and E. E. Reid, *J. Amer. Chem. Soc.*, **50**, 3062 (1928).

Sterol Metabolism. XXV. Cholesterol Oxidation by Singlet Molecular Oxygen[1]

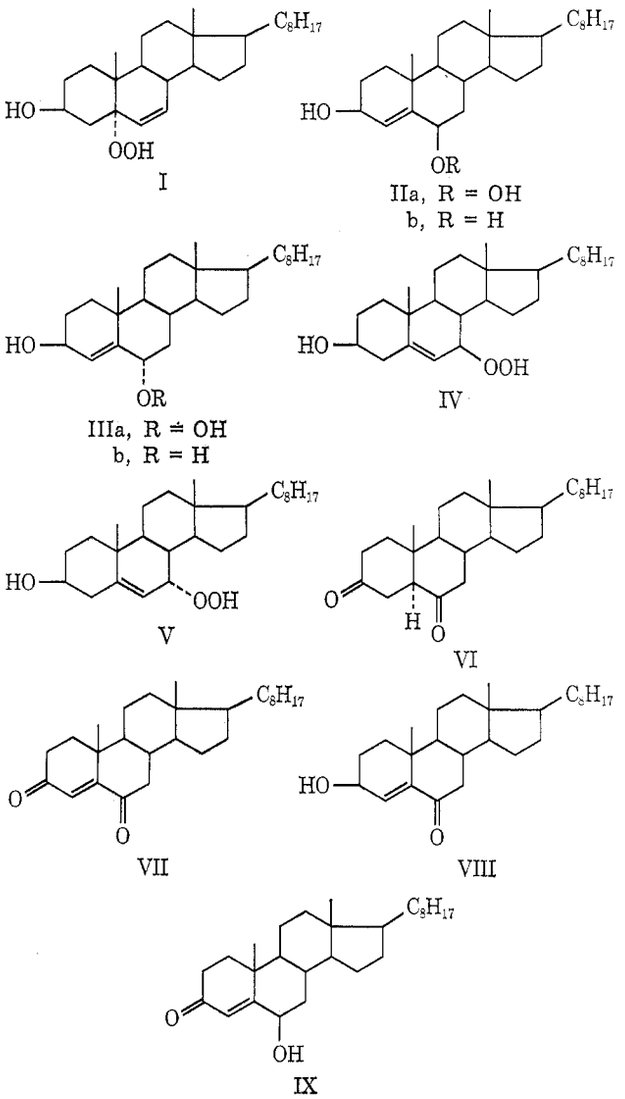
MARTIN J. KULIG[2] AND LELAND L. SMITH*

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

Received March 2, 1973

In seeking improved access to cholesterol 20α-hydroperoxide[3] implicated in the biosynthesis of pregnenolone from cholesterol[4] we examined a variety of oxidation reactions of cholesterol, among which was the well-known photosensitized oxidation in solution by excited-state (singlet) molecular oxygen. This reaction is considered to proceed by a cyclic ene mechanism with little ionic character[5] to yield 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide (I)[6] as major product. We establish herein that the epimeric 3β-hydroxycholest-4-ene 6-hydroperoxides IIa and IIIa are also formed in low yield (1–2%) but that the epimeric cholesterol 7-hydroperoxides IV and V are not formed.

The structures of the new 6-hydroperoxides IIa and IIIa were established by sodium borohydride reduction to the respective diols IIb and IIIb in the usual manner.[3] A B-ring chair conformation for IIa in which the 6β-hydroperoxide bond has quasiaxial character was supported by proton spectra in which the 6α proton appeared as a doublet of doublets coupled moderately and weakly ($J_{6\alpha,7\alpha}$ = 4.5, $J_{6\alpha,7\beta}$ = 2 Hz) to the vicinal 7α and 7β protons. Absence of strong 1,2-diaxial coupling between the 6α proton and either 7 proton together with the strong 1,3-diaxial effects of the



6β-hydroperoxide group on the chemical shift of the C-19 angular methyl group protons (shifted paramagnetically $\Delta\delta$ 0.15 ppm from their position in cholest-4-en-3β-ol[7]) additionally support this conformational assignment for IIa. Quasiaxial character for the 6β-hydroperoxide bond of IIa is thus analogous to the previously demonstrated quasiaxial character of the 6β bond of 6β-hydroperoxycholest-4-en-3-one[8] and of the 3β,6β-diol IIb.[9] The epimeric 6α-hydroperoxide IIa is accordingly the quasiequatorial epimer, a constant B-ring chair conformation being assumed for IIa and IIIa.

The quasiaxial IIa was not epimerized under reac-

(1) Paper XXIV: J. I. Teng and L. L. Smith, *J. Amer. Chem. Soc.*, **95**, 4060 (1973). Financial support of this work by the Robert A. Welch Foundation, Houston, Texas, and the U. S. Public Health Service (research grants AM13520, HE-10160, and NS-08106) is gratefully acknowledged. A preliminary account of this work has been made; *cf.* M. J. Kulig and L. L. Smith, Abstracts of Papers, 165th National Meeting of the American Chemical Society, Dallas, Texas, April 8–13, 1973, No. ORGN-078.

(2) Robert A. Welch Foundation Postdoctoral Fellow, 1971–1973.

(3) J. E. van Lier and L. L. Smith, *J. Org. Chem.*, **35**, 2627 (1970).

(4) (a) J. E. van Lier and L. L. Smith, *Biochim. Biophys. Acta*, **210**, 153 (1970); **218**, 320 (1970); (b) J. E. van Lier and L. L. Smith, *Biochem. Biophys. Res. Commun.*, **40**, 510 (1970).

(5) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **81**, 6330 (1959); **83**, 1498 (1961).

(6) (a) G. O. Schenck, *Angew. Chem.*, **69**, 579 (1957); (b) G. O. Schenck, K. Gollnick, and O.-A. Neumüller, *Justus Liebigs Ann. Chem.*, **603**, 46 (1957); (c) G. O. Schenck and O.-A. Neumüller, *ibid.*, **618**, 194 (1958); (d) G. O. Schenck, O.-A. Neumüller, and W. Eisfeld, *ibid.*, **618**, 202 (1958).

(7) Published proton spectra for cholest-4-en-3β-ol were used for comparison; see (a) I. McClenaghan and P. J. Sykes, *Chem. Commun.*, 800 (1968). (b) C. R. Narayanan and K. N. Iyer, *Tetrahedron Lett.*, 3741 (1965).

(8) J. I. Teng, M. J. Kulig, L. L. Smith, G. Kan, and J. E. van Lier, *J. Org. Chem.*, **38**, 119 (1973).

(9) The quasiaxial conformation for IIb is supported by its more mobile nature in comparison with IIIb on partition chromatographic systems in which axial alcohols generally migrate more rapidly than do equatorial alcohols. The diol IIb is more mobile than IIIb on both paper[10a] and gas–liquid (3% OV-1,[10b] 2% OV-210, and 3% SP-2401 reported herein) partition chromatographic systems.

(10) (a) L. L. Smith, *J. Amer. Chem. Soc.*, **76**, 3232 (1954); (b) J. E. van Lier and L. L. Smith, *Anal. Biochem.*, **24**, 419 (1968).

tion conditions, in solutions, or on storage as a solid, thereby differing in behavior from the analogous quasi-axial cholesterol 7α-hydroperoxide (V) which was epimerized to the quasiequatorial 7β-hydroperoxide IV under a variety of conditions.[8] The 6α-hydroperoxide IIa was also stable to conditions which epimerized the 7α-hydroperoxide V. We conclude that the allylic 6-hydroperoxides IIa and IIIa are not interconverted under the conditions of their formation and, therefore, that both IIa and IIIa are primary products formed under the experimental conditions.

Thermal decomposition of the 6-hydroperoxides IIa and IIIa on gas chromatography afforded similar but distinct patterns of pyrolysis products. Both IIa and IIIa formed their respective 6-alcohols IIb and IIIb on pyrolysis, the IIb component derived from IIa being a prominent peak on the gas chromatographic elution curve, the IIIb component derived from IIIa being in much diminished amount. The diminished yield of IIIb from IIIa reflects the relative stability of the epimeric Δ^4-3β,6-diols IIb and IIIb. The 3β,6β-diol IIb survived gas chromatography in large part whereas the 3β,6α-diol IIIb was less stable. Both Δ^4-3β,6-diols IIb and IIIb decompose on gas chromatography[10b] to two rapidly eluted minor components, one of which (t_R 0.4) may be the common dehydration product cholesta-2,4,6-triene formed pyrolytically from the epimeric cholest-5-ene-3β,7-diols, from 5α-cholest-6-ene-3β,5-diol and from I, IV, and V.[11]

Both 6β-hydroperoxides IIa and IIIa gave as major pyrolysis products 5α-cholestane-3,6-dione (VI) and cholest-4-ene-3,6-dione (VII), formed most probably by initial dehydration of the 6-hydroperoxide group to 3β-hydroxycholest-4-en-6-one (VIII), which does not survive gas chromatography. Pyrolysis of the 6 ketone VIII gave as major products the 3,6 diketones VI and VII in the same proportions as derived from pyrolysis of the 6-hydroperoxides IIa and IIIa, formation of the 5α-3,6 diketone VI and of the Δ^4-3,6 diketone VII, respectively, representing thermal isomerization and dehydrogenation reactions of VIII.

In analogy with the pyrolysis behavior of the other B-ring hydroperoxides I, IV, and V,[11] pyrolysis of the 6-hydroperoxides IIa and IIIa accordingly is rationalized in terms of formal reduction to the respective alcohols IIb and IIIb and dehydration to the common 6 ketone VIII, followed by dehydration of the product alcohols IIb and IIIb (to cholesta-2,4,6-triene) and isomerization and dehydrogenation of the 6 ketone VIII (to VI and VII, respectively). Although we have not examined the pyrolysis behavior of the related epimeric 6-hydroperoxycholest-4-en-3-ones, their close association in isolation procedures with the Δ^4-3,6 diketone VII[12] suggests that dehydration is a prominent mode of their thermal decomposition also.

The 6β-hydroxy Δ^4-3 ketone IX isomeric with VIII also formed both 3,6 diketones VI and VII as major products on pyrolysis. The two Δ^4 hydroxy ketones VIII and IX accordingly behave on pyrolysis exactly the same. Their common isomerization to the 5α-3,6 diketone VI under other thermal conditions is well known,[13] and the thermal dehydrogenations of each to the Δ^4-3,6 diketone VII find analogy in similar thermal dehydrogenations of the epimeric cholest-5-ene-3β-7-diols on pyrolysis.[11]

We have previously established that radiation-induced autoxidations of cholesterol involving radical processes lead to cholesterol 7β-hydroperoxide (IV) as major product, with the 7α-hydroperoxide V as secondary product but with no demonstrable 5α-hydroperoxide.[14] Furthermore, careful examination of these radical autoxidation product mixtures from cholesterol did not afford evidence for the presence of either 6-hydroperoxide IIa or IIIa. Moreover, neither 7-hydroperoxide IV nor V was formed in photosensitized oxygenations of cholesterol.[15] Thus, an absolute distinction between the two reaction processes obtains on the basis of different reaction products, the hydroperoxides I, IIa, and IIIa deriving from photosensitized oxygenations, the 7-hydroperoxides IV and V from radical oxidations.

The mechanism by which the 6-hydroperoxides IIa and IIIa are formed from cholesterol is of interest, the issue being whether they derive *via* attack of excited-state (singlet) molecular oxygen, *via* radical oxidation by ground-state (triplet) molecular oxygen, or *via* both pathways. Even though the 6-hydroperoxides IIa and IIIa are generated in photosensitized oxygenations in which singlet molecular oxygen is implicated, the alternative radical process could be involved, with radiation-induced homolysis of the peroxide bond of the predominant singlet molecular oxygen product 5α-hydroperoxide I leading to radical oxidation of cholesterol and formation of the 6-hydroperoxides IIa and IIIa. However, photolysis of oxygenated solutions of cholesterol in cyclohexane to which the 5α-hydroperoxide I had been added as putative radical initiator did not afford the 6-hydroperoxides IIa and IIIa as products. Rather, only traces of the radical product 7-hydroperoxides IV and V were detected. We reason that radical processes in effect in cyclohexane which formed only traces of the 7-hydroperoxides IV and V and none of the 6-hydroperoxides IIa and IIIa should also lead to formation of the 7-hydroperoxides IV and V in pyridine solution and not to the 6-hydroperoxides IIa and IIIa actually formed. Additionally we would expect of radical processes formation of equal amounts of the 6-hydroperoxides IIa and IIIa or preferential formation of the putatively more stable quasiequatorial 6α-hydroperoxide IIIa but not selective formation of the quasi-axial 6β-hydroperoxide IIa invariably formed in twice the yield of its 6α epimer IIIa. Preferential forma-

(11) J. I. Teng, M. J. Kulig, and L. L. Smith, *J. Chromatogr.*, **75**, 108 (1973).

(12) (a) A. J. Cox, *J. Org. Chem.*, **30**, 2052 (1965); (b) A. Nickon and W. L. Mendelson, *ibid.*, **30**, 2087 (1965); (c) R. Gardi and A. Lusignani, *ibid.*, **32**, 2647 (1967).

(13) (a) I. M. Heilbron, E. R. H. Jones, and F. S. Spring, *J. Chem. Soc.*, 801 (1937); (b) L. F. Fieser, *J. Amer. Chem. Soc.*, **75**, 4377 (1953).

(14) L. L. Smith, M. J. Kulig, J. I. Teng, and F. L. Hill, *J. Org. Chem.*, **38**, 1763 (1973).

(15) Our chromatographic methods developed for the purpose readily detect sterol hydroperoxides at levels less than 1% among nonperoxidic sterols; *cf.* (a) L. L. Smith and F. L. Hill, *J. Chromatogr.*, **66**, 101 (1972); (b) ref 11. Traces (much less than 1%) of IV, V, and their thermal decomposition products 3β-hydroxycholest-5-en-7-one, cholesta-3,5-dien-7-one, cholest-5-ene-3β,7α-diol, and cholest-5-ene-3β,7β-diol may be regularly detected in mother liquor fractions after recovery of unreacted cholesterol and of the hydroperoxide products I, IIa, and IIIa. Detection of these sterols in processed mother liquors cannot be taken as evidence for their formation during the oxygenation reactions, since trace amounts of these sterols can be detected in mother liquors from recrystallizations of highly purified cholesterol even though recrystallization was performed under nitrogen and in the dark.[14]

tion of the quasiaxial 6β-hydroperoxide IIa more properly resembles that outcome favored by the cyclic ene mechanism associated with attack of singlet molecular oxygen on steroidal olefins.

In oxygenated pyridine solutions of cholesterol from which both light and photosensitizer were omitted, cholesterol was recovered quantitatively without demonstrable oxidation. Inclusion of light but without added photosensitizer gave the hydroperoxides I, IIa, and IIIa in the same relative proportions but in only about 3% of the conversion yield obtained in the full, photosensitized system. Formation of I, IIa, and IIIa in the same proportions without regard to the presence of added photosensitizer suggests their formation by a common mechanism, and, in that the 5α-hydroperoxide I is an acknowledged singlet molecular oxygen product, we reason that the 6-hydroperoxides IIa and IIIa are also. The diminished level of oxidation of cholesterol to I, IIa, and IIIa in the unsensitized irradiated system may be viewed as arising from an excitation of oxygen by a process of low efficiency in which solvent pyridine act as sensitizer. In support of this view irradiated oxygenated solutions of cholesterol in cyclohexane (a solvent unlikely to act as sensitizer) without added photosensitizer afforded but traces (less than 1%) of the 7-hydroperoxides IV and V and their thermal decomposition products with no detectable 5α-hydroperoxide I or 6-hydroperoxides IIa or IIIa.

Although these arguments do not establish without reservation that the 6-hydroperoxides IIa and IIIa are products of singlet molecular oxygen attack on cholesterol, we regard them as such, and a radical origin for IIa and IIIa is clearly not supported. Formation of I, IIa, and IIIa as distinct products of singlet molecular oxygen attack on cholesterol (with I predominant) in contrast to formation of the 7-hydroperoxides[17] IV and V as radical oxidation products (with IV predominant) suggests use of cholesterol as a suitable substrate for examination of other oxidation systems, both chemical and enzymic,[16] where participation of singlet molecular oxygen or of radical autoxidation processes involving ground-state molecular oxygen is at issue.[17]

Experimental Section[18]

Photosensitized Oxidation of Cholesterol.—A solution of 1.923 g of cholesterol and 32.5 mg of hematoporphyrin in 600 ml of dry pyridine was irradiated with a Hanovia 200-W lamp (using a Pyrex glass filter) for 24 hr with a slow stream of oxygen bubbled through the irradiated solution. The volume of the reaction solution was reduced under vacuum to 50 ml and 50 ml of diethyl ether and 75 mg of decolorizing charcoal (Darco G-60) were added. The mixture was stirred for 30 min, filtered, and evaporated under vacuum at temperatures below 40°. The yellow oil thus obtained was dissolved in 50 ml of benzene, which on standing deposited 1.5520 g (74.5%) of the 5α-hydroperoxide I, mp 148–150° (lit. mp 142° dec,[5b] 148–149° dec,[5c] 145–148° dec,[5d] 149.5–150.5°,[6b] 149–151° dec[3]), identified by thin layer chromatographic and infrared spectral comparisons with an authentic sample. The yellow-colored benzene mother liquor was chromatographed on 1 mm thick Chromatoplates with chloroform–acetone (23:2) using triple ascending irrigations. The more mobile zone was eluted and identified as the 5α-hydroperoxide I. The more polar zone was eluted from the chromatoplate with acetone and rechromatographed on a 1 mm thick chromatoplate with ethyl acetate–benzene (1:1) using triple ascending irrigation thereby resolving the original polar hydroperoxide zone into two distinct hydroperoxide zones, at R_f 0.71 and 0.65.

3β-Hydroxycholest-4-ene 6β-Hydroperoxide (IIa).—Elution with acetone of the R_f 0.65 zone from the preparative thin layer chromatogram of the IIa and IIIa mixture gave 45.1 mg (2.2%) of chromatographically pure IIa. Recrystallization from benzene gave the analytical sample of IIa: mp 162–163°; $\tilde{\nu}_{max}^{KBr}$ 3350, 3125, 1600 cm^{-1}; nmr δ 0.68 (3 H, s, C-18 methyl), 0.88 (6 H, d, J = 6 Hz, C-26, C-27 methyls), 0.90 (3 H, d, J = 5 Hz, C-21 methyl), 1.20 (3 H, s, C-19 methyl), 4.21 (1 H, m, $W_{1/2}$ = 18 Hz, 3α proton), 4.34 (1 H, q, $J_{6\alpha,7\alpha}$ = 4.5, $J_{6\alpha,7\beta}$ = 2 Hz, 6α proton), 5.66 ppm (1 H, s, C-4 vinyl proton); R_f 0.65 using triple ascending irrigation with ethyl acetate–benzene (1:1); brown color with 50% aqueous sulfuric acid spray; positive Wurster red color response to *N,N*-dimethyl-*p*-phenylenediamine; pyrolysis pattern on 2% OV-210 t_R 0.40, 0.78, 1.55, 2.07 (IIb), 7.17 (VI), and 7.81 (VII); on 3% SP-2401 t_R 0.43, 0.80, 1.53, 1.99 (IIb), 6.65 (VI), and 7.13 (VIII).

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.39; H, 10.98.

Cholest-4-ene-3β,6β-diol (IIb).—A solution of 13.1 mg of IIa in methanol was reduced with an excess of sodium borohydride. After 2 hr the solution was neutralized and the product was recovered in the usual manner, yielding 9.0 mg of IIb: mp 253–256° (lit.[20] mp 254–258°); $\tilde{\nu}_{max}^{KBr}$ 3250, 1540 cm^{-1}; R_f 0.36 in ethyl acetate–benzene (1:1); blue-gray color with 50% aqueous sulfuric acid; pyrolysis pattern on 2% OV-210 t_R 0.40, 0.78, 2.07 (IIb); on 3% SP-2401 t_R 0.43, 0.80, 1.99 (IIb); identical in these properties with an authentic sample of IIb.

Material collected from preparative gas chromatography of IIa and of IIb was chromatographed on thin layer chromatoplates with ethyl acetate–benzene (1:1), and the zone with mobility of that of reference sterol IIb was eluted with acetone, recrystallized from and identified as IIb by comparison of physical properties with those of an authentic sample of IIb.

3β-Hydroxycholest-4-ene-6α-Hydroperoxide (IIIa).—Elution of the R_f 0.71 zone from the chromatogram from which IIa had initially been recovered gave 27.3 mg (1.3%) of chromatographically pure IIIa. Recrystallization from benzene gave the analytical sample of IIIa: mp 152–156°; $\tilde{\nu}_{max}^{KBr}$ 3325, 1600 cm^{-1}; R_f 0.71 in triple ascending irrigation with ethyl acetate–benzene (1:1); brown color with 50% aqueous sulfuric acid; positive Wurster red color response to *N,N*-dimethyl-*p*-phenylenediamine; pyrolysis pattern on 2% OV-210 t_R 0.40, 0.78, 1.56, 2.10 (IIIb), 7.18 (VI), 7.80 (VII); on 3% SP-2401 t_R 0.42, 0.79, 1.53, 2.03 (IIIb), 6.65 (VI), 7.13 (VII).

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.26; H, 10.97.

Cholest-4-ene-3β,6α-diol (IIIb).—A solution of 8.2 mg of IIIa in methanol was reduced with an excess of sodium borohydride, yielding after preparative thin layer chromatography 5.0 mg of IIIb: mp 174–177° (lit.[20] mp 175–179°); $\tilde{\nu}_{max}^{KBr}$ 3350, 1600 cm^{-1}; R_f 0.38 in triple ascending irrigation with ethyl acetate–benzene (1:1); yellow-tan color with 50% aqueous sulfuric acid; pyrolysis pattern on 2% OV-210 t_R 0.41, 0.78, 2.09 (IIIb); on 3% SP-2401 t_R 0.42, 0.79, 2.03 (IIb); identical in these properties with an authentic sample of IIIb.

(16) We have utilized this approach in examining the actions of soybean lipoxygenase and horseradish peroxidase on cholesterol.[1]

(17) The prior case of cholest-5-en-3-one involved competing singlet molecular oxygen attack to give 5-hydroxy-5α-cholest-6-en-3-one (as its dehydration product cholesta-4,6-dien-3-one) and radical oxidation to the epimeric 6-hydroperoxycholest-4-en-3-ones.[12b]

(18) Experimental procedures used in this study have been previously described in detail,[3] with the exceptions that proton nmr spectra were obtained using a Varian Instruments Model HR-300 spectometer operated at 60 and at 300 MHz and gas chromatography was conducted using 2% OV-210 and 3% SP-2401 columns.[11] Relative retention times (t_R) were measured *vs.* cholesterol as unit time. Preparative gas chromatography was conducted using methods previously described in detail.[19] Thin layer chromatography was conducted on silica gel HF_{254} chromatoplates irrigated with ethyl acetate–benzene (1:1). Sterol hydroperoxides were visualized with *N,N*-dimethyl-*p*-phenylenediamine;[15a] sterols were visualized afterwards with 50% sulfuric acid spray.

(19) (a) J. E. van Lier and L. L. Smith, *Biochemistry*, **6**, 3279 (1967); (b) J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **36**, 7 (1968).

(20) Physical data from J. Jacques, H. Kagan, and G. Ourisson, "Selected Constants, Optical Rotatory Power, Ia. Steroids," Volume 14 of "Tables of Constants and Numerical Data," S. Allard, Ed., Pergamon Press, Oxford, 1965, pp 438, 457, 475.

Control Oxidations.—An experiment identical with that described for photosensitized oxidation of cholesterol was run except that no photosensitizer was added. From 2.005 g of cholesterol 1.635 g of unaltered cholesterol was recovered, together with 48.4 mg of I, 1.6 mg of IIa, and 1.0 mg of IIIa, all isolated by the same means previously described. Chromatographic examination of mother liquors following recovery of I, IIa, and IIIa indicated that traces of IV, V, 3β-hydroxycholest-5-en-7-one, cholesta-3,5-dien-7-one, cholest-5-ene-3β,7α-diol, and cholest-5-ene-3β,7β-diol were present, all at much less than 1% levels. A control oxidation including photosensitizer but carried out completely in the dark gave a quantitative recovery of unaltered cholesterol.

Control oxidations carried out in cyclohexane instead of in pyridine were run similarly. A cyclohexane solution of 1.9835 g of cholesterol was irradiated for 22 hr and then processed as previously described. Crystalline cholesterol free of other demonstrable sterols was obtained in four crops (total 1.8035 g) and mother liquors after preparative thin layer chromatography gave 23.5 mg of amorphous sterols which contained IV and V in approximately 1:1 proportions but which did not contain any demonstrable IIa or IIIa. A similar control irradiation (23 hr) of 1.9753 g of cholesterol and 32.4 mg of added I was worked up in the same manner to give 1.8042 g of pure crystalline cholesterol free of other demonstrable sterols. Preparative thin layer chromatography of the mother liquors afforded 47.3 mg of sterol hydroperoxides as amorphous solids containing I, IV, and V in approximately equal proportions but which did not contain any demonstrable amounts of IIa or IIIa.

Pyrolysis Experiments.—Saturated solutions containing 2 mg of IIa, IIIa, VIII, or IX in acetone were injected onto a preparative 2% OV-210 column and material eluted from the column up to 30 min (containing minor components with t_R 0.40, 0.78, and 1.55 and IIb and IIIb if present) was collected in a capillary; material eluted between 30 and 120 min (containing VI and VII) was collected in a second capillary. Sterols were rinsed with acetone from the collecting capillaries and chromatographed on thin layer chromatoplates irrigated several times with ethyl acetate–benzene (1:1) to resolve major components. In no case did VIII or IX survive gas chromatography, both being completely converted to VI and VII.

5α-Cholestane-3,6-dione (VI).—Material eluted between 30 and 120 min from injections of IIa, IIIa, VIII, or IX was resolved by thin layer chromatography into two ultraviolet light absorbing zones at R_f 0.70 and 0.76. Material from the less mobile zone (R_f 0.70) was eluted with acetone and recrystallized from acetone, yielding pure VI: mp 169° (lit.[20] mp 168–172°); $\bar{\nu}_{max}^{KBr}$ 1715 cm^{-1}; R_f 0.70 in ethyl acetate–benzene (1:1); yellow color with 50% aqueous sulfuric acid; t_R 7.14 on 2% OV-210, 6.65 on 3% SP-2401; identical in these properties with authentic samples of VI prepared from VIII[13a] and by alkaline isomerization of IX.[13b]

Cholest-4-ene-3,6-dione (VII).—The more mobile zone (R_f 0.76) containing ultraviolet light absorbing material obtained on thin layer chromatograms from which VI had been isolated (from pyrolysis of IIa, IIIa, VIII, or IX) was eluted with acetone and recrystallized from methanol, yielding pure VII: mp 120–123° (lit.[20] mp 122–125°); $\bar{\nu}_{max}^{KBr}$ 1685, 1600 cm^{-1}; $\lambda_{max}^{CHCl_3}$ 253 nm (lit.[21] $\lambda_{max}^{CHCl_3}$ 252 nm); R_f 0.76 in ethyl acetate–benzene (1:1); yellow color with 50% aqueous sulfuric acid; t_R 7.80 on 2% OV-210, 7.13 on 3% SP-2401; identical in these properties with an authentic sample of VII.

Isomerization of 6β-Hydroxycholest-4-en-3-one (IX).—A solution of 6.3 mg of IX in 10 ml of 10% methanolic KOH was heated at 75° for 99 min. The cooled solution was neutralized and extracted into diethyl ether, the ether extract was evaporated under vacuum, and the residue was chromatographed on a chromatoplate irrigated twice with ethyl acetate–benzene (1:1). The more mobile zone (R_f 0.76) was eluted with acetone and recrystallized from methanol, yielding pure VII, mp 121–124°. The more polar zone (R_f 0.70) eluted with and recrystallized from acetone yielded pure VI, mp 166–170°, identical in physical properties with a sample of VI prepared from VIII.[13a]

Registry No.—IIa, 41209-87-4; IIb, 570-88-7; IIIa, 41209-89-6; IIIb, 15013-60-2; VI, 2243-09-6; VII, 984-84-9; IX, 570-89-8; cholesterol, 57-88-5.

(21) L. Dorfman, *Chem. Rev.*, **53**, 47 (1953).

A Ring Enlargement Reaction of Phenylmethoxycyclopropenone. A Regiospecific Mass Spectrometric CO Extrusion in Phenylmethoxycyclobutenedione

JAMES SPEROS CHICKOS

Department of Chemistry, University of Missouri—St. Louis, St. Louis, Missouri 63121

Received May 23, 1973

Isonitriles have been reported to react with small ring unsaturated ketones to give ring enlarged derivatives. Thus, 2,6-dimethylphenylisonitrile reacts with diphenylcyclopropenone (**1**) to give 4,5-bis(2,6-dimethylphenylimino)-2,3-diphenylcyclopenten-1-one (**2**), which can be hydrolyzed to diphenylcyclopentenetrione.[1] In the presence of triphenylphosphine, **1** is converted into diphenylcyclobutenedione by way of 4-(2,6-dimethylphenylimino)-2,3-diphenylcyclobuten-1-one (**3**).[2] We would like to report the results of similar experiments with phenylmethoxycyclopropenone (**4**)[3] including the structure of the intermediate 4-(2,6-dimethylphenylimino)-3-methoxy-2-phenylcyclobuten-1-one (**5**) and two interesting processes which were discovered during the course of elucidating the structure of **5**: a regiospecific loss of CO from phenylmethoxycyclobutenedione (**6**) upon electron impact and a regioselective incorporation of ^{18}O in phenylhydroxycyclobutenedione (**7**) involving the enolate ion (**7b**).

Treatment of **4** with an excess of 2,6-dimethylphenylisonitrile in refluxing benzene overnight under a nitrogen atmosphere afforded a dark solution which, after removal of the solvent and isonitrile under vacuum, slowly crystallized. Recrystallization from hexane afforded 4-(2,6-dimethylphenylimino)-3-methoxy-2-phenylcyclobuten-1-one (**5**) as yellow crystals. The gross structural features of **5** were established by hydrolysis to phenylmethoxycyclobutenedione[4] (**6**) and 2,6-dimethylaniline, isolated as the hydrochloride, in 80% yield. Compound **6** was identified by comparison to a sample prepared from squaric acid.[5] The nuclear magnetic resonance spectrum of **5** was in agreement with the proposed structure, exhibiting resonances congruous with aromatic protons (8 H), methoxyl protons (3 H), and aromatic methyls (6 H). The mass spectrum of **5**, although consistent with the proposed structure, did not give any additional structural information.

In contrast to **1**, phenylmethoxycyclopropenone (**4**) does not yield cyclopentenetrione derivatives when treated with 2,5-dimethylphenylisonitrile.[1] Carrying out the reaction at room temperature for 20 hr in the presence of trace amounts of triphenylphosphine, conditions which convert **1** into **3**,[2] afforded a mixture of

(1) T. Takizawa, N. Obata, Y. Suzuki, and T. Yanagida, *Tetrahedron Lett.*, 3403 (1969).

(2) N. Obata and T. Takizawa, *Tetrahedron Lett.*, 2231 (1970).

(3) (a) D. G. Farnum, J. S. Chickos, and P. Thurston, *J. Amer. Chem. Soc.*, **88**, 3075 (1966); (b) R. West, J. S. Chickos, and S. Osawa, *ibid.*, **90**, 3885 (1968); J. S. Chickos, L. Patton, and R. West, manuscript in preparation.

(4) E. J. Smutny, M. C. Caserio, and J. D. Roberts, *J. Amer. Chem. Soc.*, **82**, 1793 (1960).

(5) R. C. De Selms, C. J. Fox, and R. C. Riordan, *Tetrahedron Lett.*, 781 (1970).