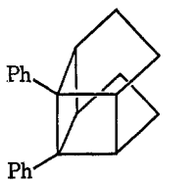
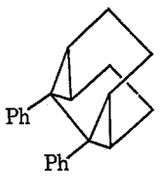


1



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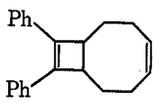

3

This reaction was neither sensitized by thioxanthone (E_T = 65.5 kcal/mol)[8] and triphenylene (E_T = 66.6 kcal/mol),[8] whose triplet energies are considered to be effectively transferred to diphenylacetylene (E_T = 62.5 kcal/mol),[8] nor quenched by diacetyl (E_T = 54.9 kcal/mol).[8] These results suggest that the addition involves singlet-excited diphenylacetylene; this is a contrast to the results that other photoreactions of diphenylacetylene proceeded *via* triplet diphenylacetylene.[2,9] This reaction is considered to involve the intramolecular photocycloaddition of the intermediate cyclobutene **3**. However, this intermediate was never observed when the photolysis was monitored by glc and uv. This can be well explained by the assumptions that the intermediate diphenylcyclobutene **3** is preferentially photoexcited on account of its large molar extinction coefficient[10] at the excitation wavelengths, and the quantum efficiency of the intramolecular reaction is greater than that of diphenylacetylene with 1,5-cyclooctadiene.

Experimental Section

Melting points are uncorrected. Ir spectra were obtained on a Hitachi EPI-S2 spectrophotometer. Uv spectra were obtained on a Hitachi 124 spectrophotometer. Mass spectra were obtained on a Hitachi RMS-4 spectrometer. Nmr spectra were taken on a high Hitachi Perkin-Elmer R-20 spectrophotometer. Glc was performed on a Simadzu GC-3AF (2 m × 3 mm, 3% SE-30 on Chromosorb W column).

Photoaddition of Diphenylacetylene and 1,5-Cyclooctadiene.—In a Pyrex vessel, a solution of diphenylacetylene (0.8 g, 0.0045 mol) in 1,5-cyclooctadiene (48 g, 0.44 mol) was irradiated for 40 hr with a 350-W high-pressure mercury lamp. After removal of the unreacted diene under reduced pressure, the remaining liquid (1.4 g) was subjected to column chromatography on Merck silica gel, 50 g (70–230 mesh). Elution in 200-ml fractions gave fractions 1–3, *n*-hexane, nil; 4–5, 5% benzene in *n*-hexane, a crystalline material. Recrystallization of this crystalline material from ethanol gave 9,10-diphenyltetracyclo[6.2.0.0^{4,10}.0^{5,9}]-decane: 924 mg (72%); mp 105.5–106.5°; ir (KBr) 3040, 3010, 2930, 1595, 1487, 1440, 750, 721, and 695 cm^{-1}; nmr (CCl_4) δ 2.0 (m, 8 H, methylene), 3.04 (br s, 4 H, cyclobutane), and 7.0 (m, 10 H, aromatic); mass spectrum *m/e* (rel intensity) 286 (1), 144 (48), 143 (100), 142 (83), 128 (39), 115 (15), and 91 (11); uv (*n*-hexane) 223 nm (ϵ 10,700), 248 (1500), 253 (920), 262 (800), and 272 (490).

Anal. Calcd for $C_{22}H_{22}$: C, 92.26; H, 7.74. Found: C, 92.25; H, 7.56.

Attempted Sensitization with Thioxanthone and Triphenylene.—Diphenylacetylene (50 mg, 0.28 mmol), 1,5-cyclooctadiene (300 mg, 2.78 mmol), and thioxanthone (10 mg, 0.047 mmol) or triphenylene (10 mg, 0.044 mmol) in benzene (3 ml) were irradiated through a liquid filter (an aqueous solution of NaBr and $Pb(NO_3)_2$, >330 nm)[11] with the 350-W high-pressure mercury lamp for 20 hr. However, the product was not observed by glc.

Attempted Quenching with Diacetyl.—Each of two quartz tubes was charged with 3 ml of a solution of diphenylacetylene (0.0337 *M*) and 1,5-cyclooctadiene (0.926 *M*) in cyclohexane. Diacetyl (44 mg, 0.512 mmol) was added to one of the tubes. The tubes were irradiated for 2 hr at 254 nm in which most of the light was absorbed by diphenylacetylene. No quenching was observed by glc.

Registry No.—**1**, 38821-22-6; diphenylacetylene, 501-65-5; 1,5-cyclooctadiene, 111-78-4.

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Sterol Metabolism. XXIII. Cholesterol Oxidation by Radiation-Induced Processes[1]

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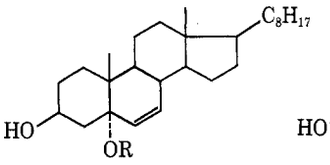
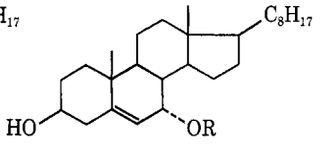
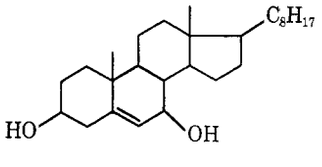
The common cholesterol oxidation products 3β-hydroxycholest-5-en-7-one (IV), cholesta-3,5-dien-7-one (V), and the epimeric cholest-5-ene-3β,7-diols (IIb, IIIb) derive by thermal decomposition of sterol hydroperoxides formed by two distinct mechanisms from cholesterol. Photosensitized oxidation of cholesterol in solution by excited-state (singlet) molecular oxygen gives 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (Ia),[3] which may rearrange in solution to the 7α-hydroperoxide IIa,[3c,4] which in turn may epimerize to the 7β-hydroperoxide IIIa.[5] Alternatively,



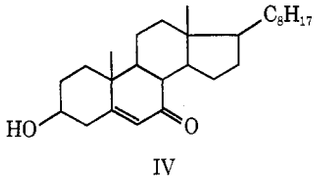
Ia, R = OH
b, R = H



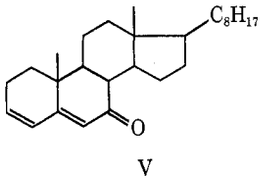
IIa, R = OH
b, R = H



IIIa, R = OH
b, R = H



IV



V

(1) Paper XXII: J. I. Teng, M. J. Kulig, and L. L. Smith, *J. Chromatogr.*, **76**, 108 (1973). Financial support of these studies by the Robert A. Welch Foundation, Houston, Texas, and the U. S. Public Health Service (Research Grants NS-08106 and HE-10160) is gratefully acknowledged.

(2) Robert A. Welch Foundation Postdoctoral Fellow, 1971–1972.

(3) (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, O.-A. Neumüller, and W. Eisfeld, *ibid.*, **618**, 202 (1958); *Angew Chem.*, **70**, 595 (1958); (d) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **81**, 6330 (1959); **83**, 1498 (1961).

(4) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 471 (1959).

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

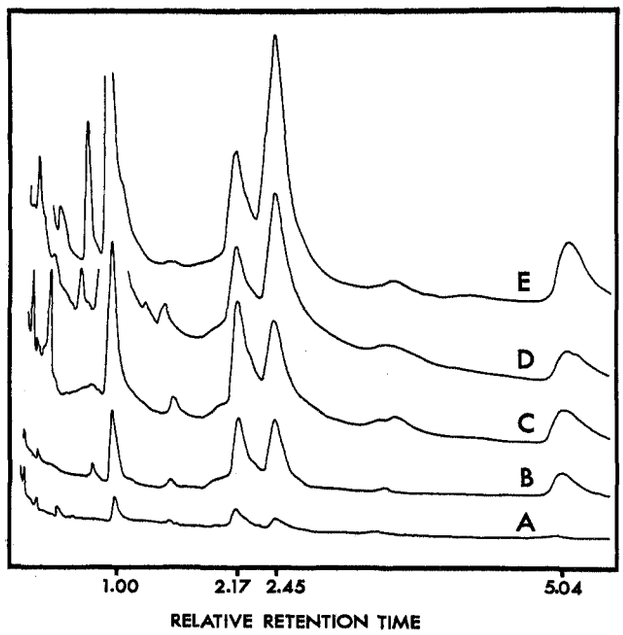


Figure 1.—Gas chromatographic (3% OV-210) detection of IIIa *via* its pyrolysis products IIIb, IV, and V (at retention times relative to cholesterol as unity of 2.45, 5.04, and 2.17 respectively): A, control; B, ^{60}Co γ radiation, 7×10^4 rad; C, 254-nm light, 1 hr; D, daylight, 6 days; E, 100° heat, 42 hr.

radical oxidation of cholesterol in solution may afford cholesterol 7-peroxy radicals or 7-hydroperoxides.[6-8] In either case the initially formed hydroperoxides give rise to the more common secondary products IIb, IIIb, IV, and V (but not Ib).

Radiation-induced oxidation of crystalline cholesterol leads to the same secondary products IIb, IIIb, IV, and V,[9] but the mechanism of their formation in the solid state has not heretofore been examined. By means of suitable chromatographic techniques[1,9d,10] (see Figure 1) we demonstrated that the initial and major sterol hydroperoxide formed from crystalline cholesterol subjected to a variety of irradiation conditions was the 7β-hydroperoxide IIIa, with small amounts of the 7α-hydroperoxide IIa formed later in the reactions. Radiations ranging from ^{60}Co γ rays through ultraviolet and visible light to infrared heat all afforded IIIa as that hydroperoxide first detected. No 5α-hydroperoxide Ia was detected. Under radiation conditions producing IIIa from cholesterol, the 5α-hydroperoxide Ia was not rearranged to the 7α-hydroperoxide IIa, nor was IIa epimerized. However, Ia, IIa, and IIIa were partially decomposed to their thermal decomposition products IIb, IIIb, IV, and V on longer exposure to radiation.

These results eliminate formation of the 5α-hydroperoxide Ia as a pathway (*via* IIa) to IIIa and accordingly participation of singlet molecular oxygen by the established cyclic ene mechanism. Furthermore, we did not detect the 7β-hydroperoxide IIIa in photosensitized oxidations of cholesterol despite a careful chromatographic examination. We thereby confirm prior findings on this point derived by less certain means.[3d] We conclude that IIIa is not a product of singlet molecular oxygen attack on cholesterol in solution or in the solid state.

Formation of IIIa from cholesterol was independent of the type of radiation used, and we consider that radical processes are implicated.[11] Initial generation of a C-7 allylic radical followed by reaction with ground-state (triplet) molecular oxygen to form a cholesterol 7-peroxy radical is supported by published electron spin resonance data.[13] Subsequent C-7 hydrogen atom abstraction by the 7-peroxy radical from another cholesterol molecule would then afford the product 7-hydroperoxides IIIa and IIa and continue the radical chain. Preferential formation of the quasiequatorial 7β-hydroperoxide IIIa in a radial process may be rationalized by consideration of the demonstrated greater thermodynamic stability of IIIa.[5] Formation of smaller amounts of IIa is thereby a random or statistically fortuitous matter. However, some preference in radical generation and attack of molecular oxygen may obtain from the crystal properties of cholesterol, for we have previously demonstrated that autoxidation of crystalline cholesterol yields 24-hydroperoxides in approximately 2:1 ratio rather than in the expected 1:1 ratio.[12a]

Autoxidation of cholesterol dispersed in aqueous sodium sterarate solutions[8a,b] similarly afforded only the 7-hydroperoxides IIa and IIIa as initially formed products, with no 5α-hydroperoxide Ia detected. Radical autoxidation of cholesterol accordingly may occur in solution, in the dispersed state, and in the solid state. The sensitive chromatographic methods used in these studies suggest anew the great ease with which highly purified cholesterol is oxidized in air. The unirradiated control (curve A of Figure 1) obtained by mere recrystallization of a highly purified cholesterol sample clearly contained the 7β-hydroperoxide IIIa, as evinced by the presence of the pyrolysis products IIIb and V on the elution curve.[14]

Access to the 7β-hydroperoxide IIIa has heretofore been *via* epimerization of IIa, in which case tedious

(6) O. Hellinger, H. Heusinger, and O. Hug, *Biophysik*, **6**, 193 (1970).

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(10) L. L. Smith and F. L. Hill, *J. Chromatogr.*, **66**, 101 (1972).

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(13) (a) W. Gordy, *Radiat. Res., Suppl. 1*, 491 (1959); (b) H. N. Rexrod and W. Gordy, *Proc. Nat. Acad. Sci. U. S.*, **45**, 256 (1959); (c) A. Ehrenberg, L. Ehrenberg, and G. Loforth, Danish Atomic Energy Commission Research Establishment, Risö, Report No. 16, 1960, cited in B. H. J. Bielski and J. M. Gebicki, "Atlas of Electron Spin Resonance Spectra," Academic Press, New York/London, 1967, pp 146–147; (d) O. Hellinger, *Biophysik*, **6**, 63 (1969).

(14) The exceptional stability of a sample of cholesterol (purified *via* the dibromide) after 34 years of storage in contact with air [*cf.* L. L. Engel and P. Brooks, *Steroids*, **17**, 531 (1971)] remains unexplained. Our chromatographic examination of the sample kindly supplied by Professor Engel failed to demonstrate the presence of any sterol hydroperoxides or secondary autoxidation products.

separation of IIIa from thermal decomposition products and from IIa was necessary. Accordingly, radiation-induced oxidation of cholesterol is of some preparative utility. Yields of 5.8–7.4% of 1,2-^{3}H-IIIa free from other detectable sterols have been attained from 1,2-^{3}H-cholesterol by irradiation with ^{60}Co γ radiation for 8 hr.

Experimental Section[15]

Radiation Conditions.—Samples (2 and 5 g) in glass beakers of crystalline cholesterol (purified to a high degree by multiple recrystallizations from methanol and in which no autoxidation component could be detected) were exposed in air to four radiation conditions. Samples were exposed to ^{60}Co γ rays in a Gammacell 200 (Atomic Energy of Canada Ltd., Ottawa) providing 2.7×10^5 rad/hr. After 15 min the 7β-hydroperoxide IIIa was readily detected. Other samples were exposed to a 254-nm germicidal ultraviolet light for 1 hr at a distance of 10 cm, after which time IIIa was readily detected. Samples were exposed to daylight and air on the laboratory bench for 6 days, at which time IIIa was readily detected. Samples were heated at 100° in an electric oven. After 42 hr IIIa was readily detected.

Sample Preparation.—Irradiated samples were dissolved (2 g/40 ml, 5 g/100 ml) in the dark at 40° under N_2 in diethyl ether–methanol (1:1). Chilling to 5° yielded crystalline cholesterol which was filtered off for analysis. The mother liquor was concentrated under vacuum to incipient crystallization, and a second crop of crystalline cholesterol was removed. Concentration under vacuum was repeated until the mother liquor volume was 5 ml (for 2-g samples) or 10 ml (for 5-g samples). The concentrated mother liquor was preparatively chromatographed on 0.25 mm chromatoplates of silica gel HF_{254} using benzene–ethyl acetate (17:8) in triple ascending irrigations. The sterol hydroperoxide zone was located and eluted from the chromatoplate with 5–10 ml of acetone, the acetone was removed under vacuum, and the sterol residue was redissolved in 100 μl of acetone for analysis.

Replicate experiments were handled by a more direct method. The mother liquor obtained by crystallization of cholesterol and concentration was evaporated under vacuum and the sterol residue was subjected to analysis without intermediate preparative thin layer chromatography. Essentially identical results were obtained by the two different sample preparation methods.

Sample Analysis.—Mother liquor sterols in 100 μl of acetone were subjected to thin layer chromatography with up to 100–200 μg of total sterols applied to the chromatoplate per analysis. Reference sterols were run on the same chromatoplate. Each sample was analyzed as such and also after reduction on the chromatoplate with 20 μl of a 10% sodium borohydride solution in methanol.[17] In that no 7-ketone IV was detected in these samples by ultraviolet light absorption of the chromatoplate reduction with borohydride gave product alcohols solely from the sterol hydroperoxides present. Each chromatoplate was visualized with *N,N*-dimethyl-*p*-phenylenediamine and 50% sulfuric acid for identification with confidence of each detected sterol component. In each case IIIa was the first sterol product to be detected in irradiated samples, with IIa forming more slowly and at much reduced levels. No Ia could be detected as such or as the reduced product Ib.

Samples of mother liquor (2–10 μl) were analyzed by gas chromatography on SP-2401 and OV-210 columns at the same time.[1] Uniform and characteristic elution curves were obtained for all samples, in which IIIa and V predominated. Typical elution curves are given in Figure 1 in which 3% OV-210 columns were used. Key relative retention times noted are 1.00, reference cholesterol; 2.17, VI; 2.45, IIIb; 5.04, IV. Cholesta-4,6-dien-3-one would appear at 3.46 and IIIb at 2.27.

Stability Experiments.—Pure samples of Ia, IIa, and IIIa were exposed to the same irradiation conditions. In the case of ^{60}Co γ radiation, exposure times of 30 min were also used. Analysis of these samples by both thin layer and gas chromatography established that thermal decomposition only had occurred, with no evidence of conversion of Ia to IIa, of IIa to IIIa, or of conversion of IIIa to other hydroperoxides.

1,2-^{3}H-Cholesterol 7β-Hydroperoxide (IIIa).—An aliquot (*ca.* 10 μCi) of 1,2-^{3}H-cholesterol was chromatographed on 0.25 mm thick silica gel HF_{254} chromatoplates irrigated three times with benzene–ethyl acetate (17:8), and the eluted radioactive cholesterol was rechromatographed a second time. Dilution with 500 mg of crystalline highly purified carrier cholesterol gave a sample assaying 29,700 dpm/mg. A portion of this material (200 mg) in a small glass vial open to the air was irradiated with ^{60}CO γ radiation for 8 hr, after which time the irradiated sample was dissolved in 200 ml of methanol, chilled overnight, and the resultant crystalline 1,2-^{3}H-cholesterol filtered. The solvent was evaporated under vacuum, and the sterol residue was dissolved in a minimum volume of acetone and chromatographed on 0.25 mm thick silica gel HF_{254} chromatoplates using benzene–ethyl acetate (17:8) with triple irrigation in the usual fashion. The IIIa zone was located and excised from the chromatoplate. The 1,2-^{3}H-IIIa was eluted with acetone. Rechromatography of the material twice more using the same system sufficed to give pure 1,2-^{3}H-IIIa free from IIa and other detectable sterols. Only one component (1,2-^{3}H-IIIa) was detected on thin layer chromatograms or on gas chromatography on 2% SP-2401. Sodium borohydride reduction gave only one radioactive component identified as IIIb, with no other detectable sterols present. The radioactive IIIa was dissolved in 1 ml of acetone, and 50-μl aliquots were assayed for radioactivity to determine yields of 5.8 and 7.4% for two separate preparations.

Registry No.—Cholesterol, 57-88-5.

(15) Solvents were redistilled prior to use. All sterols used were of high purity as judged by melting point, infrared absorption spectral, and thin layer and gas chromatographic criteria. Thin layer chromatography was conducted on 0.25 mm thick 20 × 20 cm chromatoplates of silica gel HF_{254} (E. Merck GmbH., Darmstadt) using benzene–ethyl acetate (17:8) and triple ascending irrigation by techniques previously described in detail.[9d] Typical mobility data with IIIa serving as unit mobility follow: IIIa, 1.00; IIa, 0.91; Ia, 0.91; IIb, 0.53; IIIb, 0.60; Ib, 0.76. Sterol hydroperoxides were detected by *N,N*-dimethyl-*p*-phenylenediamine used as a spray.[10] Sterols were also detected by their characteristic colors developed with 50% aqueous sulfuric acid used as a spray.[9d]

Gas chromatography was conducted by procedures previously described in detail,[16] but using 2–3% SP-2401 and 2–3% OV-210 liquid phases on 100–120 mesh Supelcoport (Supelco Inc., Bellefonte, Pa.) for the confident resolution of IIb and IIIb.[1] Retention data for the several sterols involved in this study were essentially the same as those previously reported.[1]

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The Stereoselectivities of Lithium Aluminum Trialkoxyhydrides

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The modification of lithium aluminum hydride ($LiAlH_4$) by the addition of various alcohols (or ketones), and subsequent use of the resulting lithium aluminum alkoxyhydrides (1) in the reduction of the model system dihydroisophorone (2), has led to two basic conclusions.[1] First, lithium aluminum alkoxyhydrides are generally more highly stereoselective than $LiAlH_4$ itself, presumably because of the greater bulk of the alkoxyhydride reagents. It was recognized[1,2] that certain alkoxyhydrides, such as lithium aluminum tri-*tert*-butoxyhydride, were less stereoselective than their apparent bulk suggested. This was explained by Ashby and coworkers,[3] who showed that, while the

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