REACTIVITY AND GEOMETRY IN ALLYLIC SYSTEMS

III. PHOTOSENSITIZED OXYGENATION OF CHOLEST-4-ENE, A STEROID OLEFIN WITH A CONFORMATIONALLY FLEXIBLE RING[1,2]

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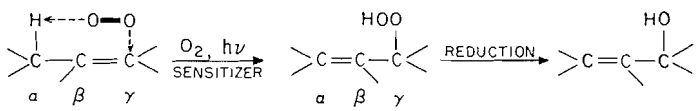
Dedicated to Professor R. B. Sandin on the Occasion of his Sixty-Eighth Birthday

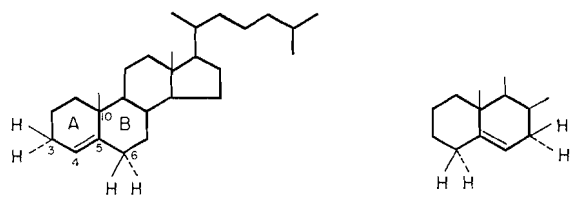
ABSTRACT

Photosensitized oxygenation of cholest-4-ene with hematoporphyrin in pyridine followed by reduction of the derived hydroperoxides gave five allylic alcohols in these relative amounts: cholest-4-en-3α-ol (V*a*, 37%); cholest-4-en-3β-ol (IV*a*, 7.5%); 5α-cholest-3-en-5-ol (VIII*a*, 29%); 5β-cholest-3-en-5-ol (VI*a*, 2%); and cholest-5-en-4β-ol (X*a*, 8.5%). A by-product from breakdown of the hydroperoxides was cholest-4-en-3-one (III, 6.5%). The Δ^4-stenols are not primary products from the photosensitized process but arose by gradual isomerization of the initially derived 5-hydroperoxycholest-3-enes. When the original hydroperoxide mixture was allowed to stand in chloroform, the rearrangement went to completion and no isolable amounts of the Δ^3-stenols remained. After rearrangement, V*a* and IV*a* were present in a ratio of about 8:1, and this sequence affords a convenient new route to cholest-4-en-3α-ol. The photooxidation products and their relative proportions are readily understood if oxygen can attack both half-chair conformations of ring A in cholest-4-ene and can cyclically abstract a quasi-axial (or an axial) allylic hydrogen. Conclusions are drawn on the importance of steric hindrance and of ring inversion on the photosensitized oxygenation reaction.

INTRODUCTION

Photosensitized oxygenation of monoolefins gives allylic hydroperoxides with a rearranged double bond (1). Work with steroids showed that the bonds formed and broken (C—O and C—H respectively) are *cis* to each other and led to the suggestion of a cyclic mechanism, with no implication about the timing of the events or the extent of participation by the sensitizer (2, 3). Geometric considerations indicate that in a half-chair cyclohexenoid ring a quasi-axial (*a'*) hydrogen is better suited than a quasi-equatorial





I II

(*e'*) hydrogen for cyclic abstraction. All results so far are interpretable on this basis and no definitive examples have been found of *e'* hydrogen abstraction in a rigid ring system.

[1]*For Part II see ref. 15a.*
[2]*Taken from the Ph.D. Dissertation of W. L. Mendelson, Johns Hopkins University, 1963.*
[3]*Alfred P. Sloan Foundation Fellow, 1957–61.*

In those cases where the preferred ring conformation is different for the starting olefin and product, ring inversion or distortion must occur at some stage, but in one test case studied (5β-cholest-3-ene) it was shown that this factor by itself was insufficient to stop the reaction, though it may have slowed it (4). Results with steroids and simpler olefins indicate that the reaction is subject to steric hindrance. In particular, an axial alkyl group in a 1,3-relationship both to the developing C—O bond and to the allylic C—H bond inhibits the oxygenation markedly (2–5).[4] Which of these two 1,3-interactions is the more important emerges from the present study and from work to be described elsewhere (4, 5).

Although several monocyclic olefins have been photooxygenated with sensitizers (1), the only studies involving complete product analysis have been by Kenney and Fisher (6, 7) and by Schenck *et al.* (8). The former workers examined (+)-carvomenthene, and the latter group investigated (+)-carvomenthene and (+)-limonene. Both research groups satisfactorily accounted for their products on the basis of the cyclic *cis* mechanism.[5] To find out the effect of conformational flexibility in a condensed ring system we examined the photosensitized oxygenation of cholest-4-ene (I). The double-bond environment in this substrate is similar to that in Δ^5-steroids (part of structure II) but, unlike these ring B olefins, cholest-4-ene can exist in two interconvertible half-chair conformations; hence, it was of interest to learn if products could be derived from each. Furthermore, the behavior of the cholest-4-ene system would help assess other steric features of the reaction.

RESULTS

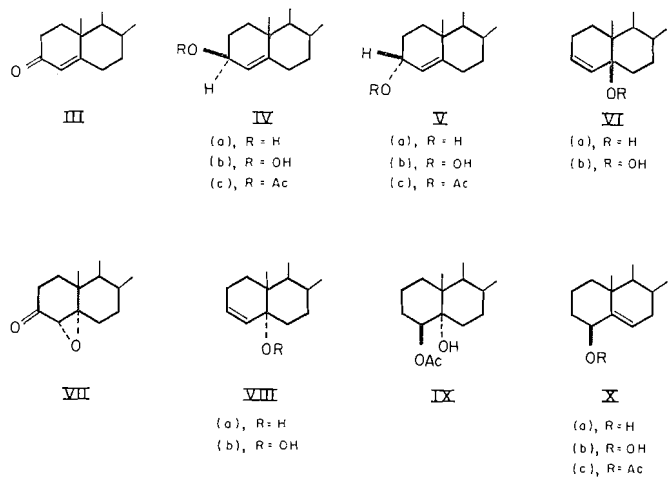
Cholest-4-ene was prepared from cholest-4-en-3β-ol acetate (or benzoate) and also from cholest-4-en-3-one (III) by reported methods (9, 10). To facilitate identification and assay of oxygenation products we separately synthesized the five allylic alcohols IV*a*, V*a*, VI*a*, VIII*a*, and X*a*, and some of their esters as follows.

Cholest-4-en-3β-ol (IV*a*) was obtained by reduction of III with lithium aluminium hydride and then separation of the epimers with digitonin. In a simplified route sodium borohydride reduction produced the 3β-ol in such preponderance that this epimer could be isolated directly by crystallization without resort to digitonide formation. Reduction of III with aluminium isopropoxide (11) gave the 1:1 complex of the epimers IV*a* and V*a*. After removal of the 3β-epimer with digitonin we obtained cholest-4-en-3α-ol (V*a*). An improved route to V*a* arose from the photooxygenation of I and will be described in the sequel. The reduction of 4β,5-epoxy-5β-cholestan-3-one with hydrazine gave us 5β-cholest-3-en-5-ol (VI*a*) as reported (24), and we applied the same reduction to 4α,5-epoxy-5α-cholestan-3-one (VII) and obtained the new allylic alcohol 5α-cholest-3-en-5-ol (VIII*a*). As expected (12) the molecular rotation increment of VIII*a* relative to 5α-cholest-3-ene for introduction of the OH group at C-5 is strongly negative. The synthesis of 4β-acetoxycholest-5-ene (X*c*) by a reported method proved unsuitable; hence, we developed a two-step procedure which involved conversion of cholest-4-ene to the diol monoacetate IX by treatment with hydrogen peroxide in acetic acid – acetic anhydride, followed by dehydration to X*c*.

We photooxygenated cholest-4-ene (I) in dilute pyridine solution (ca. 0.03 *M*) at

[4]*The formation of relatively little cis-carveol (5%) among the photosensitized oxygenation products of (+)-limonene can be rationalized on a similar basis, although alternate explanations are possible (8).*

[5]*Kenney and Fisher originally favored a concerted process for the cyclic mechanism (6) but have reinterpreted their results (7) in terms of Schenck's stepwise mechanism (1) in which* C—O *bond formation creates an intermediate diradical and precedes* C—H *cleavage.*



about 35–40° with hematoporphyrin (ca. 2×10^{-4} *M*) as sensitizer. In a typical run it underwent about 90% conversion in 25 h,[6] and the crude product (after correction for unchanged olefin) contained 6.5% cholest-4-en-3-one (III, assayed by its ultraviolet absorption at 240 mμ) and 83% mixed hydroperoxides (assayed by quantitative hydrogenation of an aliquot). The total product was reduced with sodium iodide and chromatographed on alumina. In addition to starting olefin (ca. 11%) and cholest-4-en-3-one (III) we identified five allylic alcohols IV*a*, V*a*, VI*a*, VIII*a*, and X*a*. Table I lists the alcohols, in order of elution, and their approximate relative yields after correction for recovered olefin. The compounds were identified by infrared spectroscopic comparison and, except for VI*a*, also by appropriate melting point and mixture melting point comparisons of the alcohol (or one of its esters) with the genuine samples from synthesis. In a control photooxygenation conducted in the same way except that the sensitizer was omitted, no detectable hydroperoxidic material was produced and the starting olefin was recovered entirely. When a free-radical initiator (*t*-butyl hydroperoxide) was added in nearly equivalent amount, a control run in the absence of sensitizer gave about 5% oxidation. These results reasonably exclude a radical-chain pathway as a significant source of oxygenation products.

TABLE I*

	Relative %
5α-Cholest-3-en-5-ol (VIII*a*)	29
Cholest-5-en-4β-ol (X*a*)	8.5
5β-Cholest-3-en-5-ol (VI*a*)	2
Cholest-4-en-3α-ol (V*a*)	37
Cholest-4-en-3β-ol (IV*a*)	7.5

*The total recovery from chromatography was 95%, which included about 9.5% of unidentified mixtures.

DISCUSSION

Because photosensitized oxygenation is invariably accompanied by a shift of the double bond (1), the hydroperoxides IV*b* and V*b* (corresponding to the alcohols IV*a* and V*a*)

[6]*Longer reaction times encouraged breakdown of the hydroperoxides, as evidenced from diminished infrared intensities in the* OH *region and increased intensities in the carbonyl region.*

cannot be primary products of the sensitized process. That they can arise by subsequent allylic rearrangement of the expected 5-hydroperoxycholest-3-enes in the original solution or during the iodide reduction was indicated when a chloroform solution of the crude hydroperoxidic mixture was found to mutarotate during polarimetric readings. When rearrangement in the chloforom solution was allowed to go to completion (48 h), the mixture was reduced with sodium iodide and chromatographed as before. The two cholest-3-en-5-ols (VI*a* and VIII*a*) were no longer present, and the epimeric cholest-4-en-3-ols (V*a* and IV*a*) were obtained in higher yields and in a ratio greater than 8:1 (cf. original ratio of 5:1 from Table I). These allylic rearrangements of tertiary hydroperoxides to secondary hydroperoxides have analogy,[7] and, because of the favorable final proportion of the α-epimer V*a*, we recommend photosensitized oxygenation of cholest-4-ene followed by allylic rearrangement of the hydroperoxidic mixture in chloroform as a convenient route to cholest-4-en-3α-ol (V*a*). From the *cis* stereochemistry required both for the oxygenation step (2, 3) and for the subsequent allylic rearrangement of the tertiary hydroperoxides (13, 14) it follows that the combined yield (66%) of V*a* and VIII*a* represents the total abstraction of the α-hydrogen at C-3; similarly, the combined yield (9.5%) of IV*a* and VI*a* represents the net abstraction of the β-hydrogen at C-3. The fifth alcohol X*a* (obtained in 8.5% yield) arises from removal of the β-hydrogen at C-6. These are the first cases where β-attack has been observed in photosensitized oxygenation of a steroid monoolefin. The relative ease of hydrogen abstraction for $3\alpha/3\beta/6\beta$ lies between 8.5/1.1/1 and 7.8/1.9/1.[8] The following conformational analysis satisfactorily accounts for these results and leads to some new conclusions about the oxygenation reaction.

In cholest-4-ene, ring A can adopt two half-chair conformations depicted in XI and XII. We shall first consider attack by oxygen[9] at C-5 and abstraction of hydrogen from C-3. In half-chair XI, α-attack by oxygen meets no serious steric hindrance and the 3α-hydrogen is quasi-axial (*a'*). Consequently, circumstances are favorable for production of hydroperoxide XIII by a cyclic mechanism. Importantly, this hydroperoxide can adopt only one half-chair conformation (shown in XIII) and no ring inversion is involved when the double bond shifts from Δ^4 (in XI) to Δ^3 (in XIII). The C—O bond in XIII is *a'* and therefore well disposed sterically and stereoelectronically for intramolecular allylic rearrangement to XV, which can ultimately equilibrate with its alternate half-chair conformation, XVII.

Beta attack by oxygen cannot be consummated from half-chair XI because the 3β-hydrogen is quasi-equatorial (*e'*) and therefore relatively inaccessible for cyclic transfer.[10] The situation changes in half-chair XII, because now the C—H bonds in ring A have reversed their conformational character. The 3α-hydrogen is *e'* and precludes hydroperoxidation from the α-side.[11] On the other hand, the 3β-hydrogen is *a'* and the overall steric situation for β-attack is now similar to that noted above for α-attack on half-chair

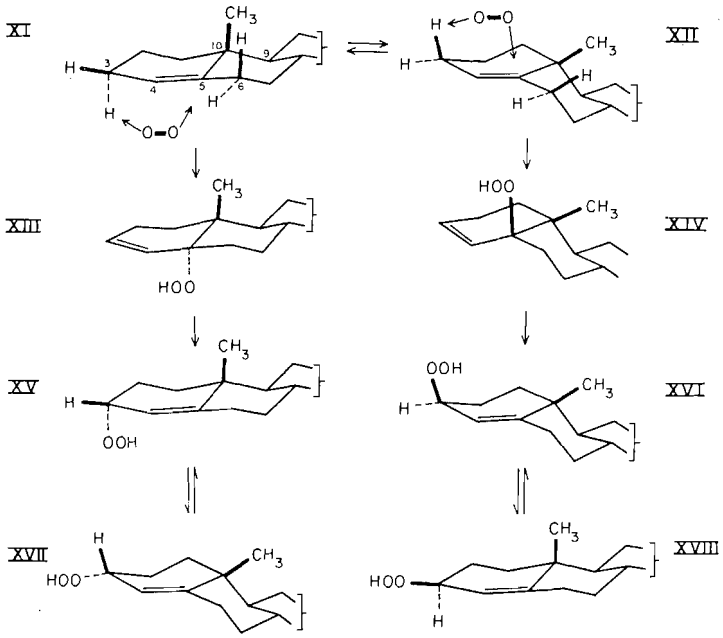
[7]*Schenck et al. (13) and Lythgoe and Trippett (14) first observed this type of isomerization with 5-hydroperoxy-5α-cholest-6-en-3β-ol, which rearranged to 7α-hydroperoxycholest-5-en-3β-ol. Schenck's mechanistic studies showed that the isomerization is both intramolecular and stereospecifically cis.*

[8]*The uncertainty in these ratios arises because a portion of the 3α- and 3β-hydroperoxides breaks down to cholest-4-en-3-one, and presently we have no way to estimate how much of the ketone is derived from each hydroperoxide. The first set of figures applies if the ketone (6.5%) arose exclusively from Vb; the second set applies if the ketone came entirely from IVb. The true figures probably lie between these extremes.*

[9]*The photochemical aspects of sensitized reactions have been discussed elsewhere (see refs. 1, 3, and 15 for leading references).*

[10]*Note also that the angular methyl group at* C-10 *would present an almost eclipsed 1,2-interaction to an incipient beta* C—O *bond at* C-5 *and might further impede β-attack.*

[11]*An added feature that could impede α-attack on XII is the* C_9—C_{10} *bond, which would eclipse a* C—O *bond being created at* C-5.



XI. On this basis, hydroperoxide XIV should arise from half-chair XII about as readily as XIII did from half-chair XI. Once formed, XIV has its hydroperoxy group suitably poised (*a'* to ring A) for allylic rearrangement to XVI. Ring inversion plays no role in the formation of XIV or in its isomerization to XVI but enters only in the final equilibration of XVI with its alternate half-chair form XVIII. The yields in Table I reveal that the fraction of XIV that rearranged (0.83 ∓ 0.04)[8] is higher than that (0.58 ± 0.02)[8] of XIII. The A/B *cis* ring fusion in XIV is probably less stable than the A/B *trans* fusion in XIII, and the drive to release strain may be correspondingly greater in the former system.

We now turn our attention to oxygenation at C-4 accompanied by hydrogen abstraction from C-6. Alpha attack is not fruitful from either half-chair XI or half-chair XII, because the 6α-hydrogen is equatorial (*e*) to ring B and unsuitably oriented with respect to the olefinic bond. Beta attack on XI is sterically blocked by the methyl group at C-10, which is axial to ring B and quasi-axial to ring A and which presents diaxial-type 1:3-interactions to a developing C—O bond at C-4 and also to the 6β-hydrogen. Other examples that attest to the difficulty of hydroperoxidation in molecules with comparable steric situations are available (2–8). In half-chair XII the offending methyl group is *e'* to ring A and no longer confronts an incipient beta C—O bond at C-4 with serious hindrance. Consequently, the cholest-5-en-4β-ol (X*a*) is most reasonably derived from half-chair XII and, concomitant with the double-bond shift from Δ^4 to Δ^5, ring A must either undergo conformational inversion or develop initially into a twist boat form. Another example where the ring-inversion factor has failed to block oxygenation has been observed with 5β-cholest-3-ene (4).

Half-chair XII contains a geometric feature not exemplified so far. The angular methyl group is quasi-equatorial to ring A but is axial to ring B and presents a diaxial 1:3-interaction to the 6β-hydrogen but not to a developing beta C—O bond at C-4. Therefore, unless X*a* is formed by some abnormal mechanism, this case illustrates that a diaxial 1:3-relationship between an alkyl group and an allylic hydrogen is insufficient by itself to block

the reaction. Insofar as these hindrance effects can be separately evaluated, steric interactions with the developing C—O bond rather than with the allylic hydrogen appear to be of greater importance. The formation of X*a* from cholest-4-ene is of additional interest, because the double bond is initially exo to ring B and finally exo to ring A. Schuller and Lawrence have implied that a structural change of this type would involve a prohibitive amount of strain as the transition state is approached and would lead to nonreactivity (16).

EXPERIMENTAL

General

Melting points are corrected. Optical rotations refer to the sodium D-line and were taken at room temperature in chloroform. Ultraviolet spectra were taken in 95% ethanol on a Beckmann DK-2 or a Cary model 11 M recording instrument. Infrared spectra were taken in carbon disulfide solution with a Perkin–Elmer model 21 double-beam spectrophotometer. In color tests for hydroperoxide 1 drop of a saturated solution of sodium iodide in isopropyl alcohol was added to the unknown (0.002–0.003 g) dissolved in glacial acetic acid. Authentic steroid hydroperoxides were used as controls.

To assay for total hydroperoxide in a crude product we used quantitative hydrogenation with Raney nickel catalyst (17), in pyridine (13, 18). The hydrogen uptake was usually complete within 20–30 min with pre-blanked catalyst and was reproduced in a duplicate run to within 3–4%. For the determinations we used 0.015–0.030 g of the hydroperoxide and ca. 0.5 g of the ethanol-moistened Raney nickel.

Reductions of hydroperoxides with sodium iodide were conducted as soon as possible after work-up of the oxygenation. In a typical reduction, sodium iodide (ca. 0.6 g) was dissolved in a solution containing the crude hydroperoxide (0.15 g) in absolute methanol (10 ml), anhydrous ether (2 ml), and glacial acetic acid (3–4 drops). After 12 h most of the solvent was removed, an excess of ether was added, and the solution was washed with sodium thiosulfate (until the ether was clear) and then with water. The ether was dried over sodium sulfate, which was the drying agent throughout this work, and evaporation gave the allylic alcohol.

Sterols were acetylated and benzoylated by conventional procedures (3).

Photosensitized oxygenations were conducted in a vertical pyrex tube (30 cm) irradiated along its length by two standard fluorescent desk lamps (each containing two 15-W fluorescent tubes, each about 17 inches long) mounted about 1 inch away. A long glass tube with a fritted glass plate at the end was used to admit oxygen at the bottom of the solution, slowly and without interruption. The lamps kept the temperature of the solution between 35–40°. Hematoporphyrin (Mann Research Laboratories) and reagent grade pyridine were used in all of the photosensitized oxygenations. A typical work-up procedure for a 20-ml aliquot was as follows. After evaporation of the solvent under reduced pressure at 35–45°, anhydrous ether (15 ml) and charcoal (ca. 0.02 g) were added; the mixture was warmed briefly and filtered, and the filtrate was evaporated under vacuum at a temperature not exceeding 40°.

Cholest-4-en-3-one (III)

This compound, prepared as described (19), had m.p. 79–81°, $[\alpha]$ +90°, λ 241 (ϵ 16 600). Reported m.p. 79.5–80.5° (19), λ 240.5 (ϵ 18 000) (20).

*Cholest-4-en-3β-ol (IV*a*)*

(i) By Reduction with Lithium Aluminium Hydride

Reduction of cholest-4-en-3-one (III) with lithium aluminium hydride yielded a white solid, usual m.p. 125–127°. The optical rotations (three runs) ranged from +55° to +65°, which indicated about a 4:1 ratio of cholest-4-en-3β-ol to cholest-4-en-3α-ol (V*a*). Recrystallization from aqueous ethanol or methanol–acetone raised the m.p. to 129–130°. In contrast to a literature report (21*a*), the 1:1 molecular compound of IV*a* and V*a* (m.p. 141°, $[\alpha]$ +85°) could not be obtained by us this way (cf. 21*b*). However, we obtained pure IV*a* (m.p. 132–133°, $[\alpha]$ +46°) by treatment of the reaction product with digitonin as reported (21). We could not isolate cholest-4-en-3α-ol (V*a*) from the digitonide mother liquors, presumably because it was present only in small amounts.

(ii) By Reduction with Sodium Borohydride

A suspension of sodium borohydride (1.2 g) in a small volume of methanol was added to the enone (4.1 g) in methanol (300 ml) and the mixture was stirred at room temperature for 24 h, was poured into water (300 ml), and was extracted with ether, which was dried and evaporated. The product was crystallized once from ethyl acetate – methanol, m.p. 126–127° (3.5 g), $[\alpha]$ +51°. Pure cholest-4-en-3β-ol (IV*a*, m.p. 130–132°, $[\alpha]$ +46°) was obtained by one or two further recrystallizations (ca. 75% recovery) from ethyl acetate – methanol or from aqueous ethanol. Reported m.p. 131–132°, $[\alpha]$ +44° in benzene (21).

Acetylation of IV*a* gave cholest-4-en-3β-ol acetate IV*c*, m.p. 84–86°, $[\alpha]$ +6°. Reported m.p. 87–88°, $[\alpha]$ +8° (22). Benzoylation of IV*a* gave cholest-4-en-3β-ol benzoate, m.p. 125–126°, $[\alpha]$ +1°. Reported m.p. 125–128°, $[\alpha]$ ±0° (9).

Cholest-4-en-3α-ol (Va)

Reduction of enone III with aluminium isopropoxide (11) gave us the 1:1 complex of IV*a* and V*a* with m.p. 141–142°, [α] +84°. Separation of this complex with digitonin (21*a*) gave cholest-4-en-3α-ol (V*a*) as a hardened oil ([α] +121°). Two crystallizations from acetone–water gave solid with m.p. 78–82°. Reported m.p. 84°, [α] +120.8° in benzene (21).

Acetylation of V*a* (oil) gave cholest-4-en-3α-ol acetate V*c*, m.p. 83–84°, [α] +175°. Reported m.p. 82–83°, [α] +177° (22).

Cholest-4-ene (I)

Method A

This method involved the lithium–ethylamine reduction of the allylic acetate (IV*c*) (or benzoate) as reported (9). The product was purified by chromatography on alumina and then by one crystallization from ethyl acetate – methanol, m.p. 81–82°, [α] +73°. Reported m.p. 82–83.5°, [α] +76°, for olefin purified through the dibromide (20). The infrared spectrum (potassium bromide disc) of our product had bands at 1 655 and 810 cm^{-1} in agreement with those reported by Bladon *et al.* (20). The reduction proceeded equally well (yields 35–40%) with either the acetate or benzoate. In one run we carried out the reaction on a mixture (ca. 1:2) of cholest-4-en-3α-ol and -3β-ol acetates and obtained cholest-4-ene of the same purity.

Method B

This method was useful for larger scale preparations of the olefin (2.5 g); however, the product was not as pure as that from method A. This procedure involved treatment of an ether solution of the cholest-4-en-3-one (II) with aluminium chloride and lithium aluminium hydride as reported by Wheeler and Mateos (10). They reported m.p. 65°, [α] +56°, for material obtained after chromatography of the crude product and indicated that the product was a mixture of cholest-4-ene (80%) and cholestane (20%). Our product, after chromatography, had m.p. 65–70°, [α] +56°. Two crystallizations from ethyl acetate – methanol gave cholest-4-ene (25%, m.p. 78–80°, [α] +70°), whose infrared spectrum (potassium bromide) was identical in all essential respects with that of the olefin produced by method A.

4β,5-Epoxy-5β-cholestan-3-one

This synthesis was carried out by modification of the procedure of Plattner *et al.* (23). A solution of cholest-4-en-3-one (2.0 g) in 95% ethanol (500 ml, at room temperature) was treated simultaneously and dropwise with 30% hydrogen peroxide (12 ml) and 5 *N* sodium hydroxide (12 ml). After the solution had stood at 0° for 24 h, water (100 ml) was added and most of the solvent was removed in a rotary evaporator. The resulting pasty solution was extracted with methylene chloride, and the organic layer was washed several times with water and dried over sodium sulfate. Evaporation of the solvent gave a solid, which crystallized in needles from acetone–methanol, 1.3 g, m.p. 118–119°, [α] +128°. Reported m.p. 116–117°, [α] +136° (23).

5β-Cholest-3-en-5-ol (VIa)

This compound was obtained by treatment of 4β,5-epoxy-5β-cholestan-3-one with hydrazine hydrate as reported (24). The crude product (oil, 0.25 g) was chromatographed on alumina (9 g). Elution with benzene – petroleum ether yielded a clear oil (0.15 g, [α] +94°) which hardened to a semisolid. Reported m.p. 93–94.5°, [α] +98°. The infrared spectrum of our product had bands at 3 600, 3 550, 1 015, 1 000, 975, 925, 740, 720, and 625 cm^{-1}.

4α,5-Epoxy-5α-cholestan-3-one (VII)

This epoxyketone was prepared by the photosensitized oxygenation of cholest-4-en-3β-ol IV*a* as reported previously (15*a*). After chromatography on deactivated alumina and crystallization, it had m.p. 121–122°, [α] −39°. Reported m.p. 120–121°, [α] −42.5° (26).

5α-Cholest-3-en-5-ol (VIIIa)

This new allylic alcohol was prepared by treatment of 4α,5-epoxy-5α-cholestan-3-one (VII) with hydrazine hydrate (24) followed by chromatography on alumina. Elution with benzene – petroleum ether (1:1) gave a solid, m.p. 72–74°, with infrared bands at 3 600, 955, 930, 918, 895, 725, and 688 cm^{-1}. One crystallization from methanol gave crystals, m.p. 74–75°, [α] −14°. (For analysis, the material from the oxygenation of cholest-4-ene was purified (see below).)

4β-Acetoxy-cholest-5-ene (Xb)

The following procedure was found to be superior to a reported method (25). A solution of cholest-4-ene (0.5 g) in benzene (1 ml) was treated with acetic anhydride (3 ml) and acetic acid (8 ml). The reaction mixture was stirred rapidly at 40–45° while 30% hydrogen peroxide (3 ml) was added over a 2.5-h period. Stirring was continued for 14 h at 40–50°, after which several milliliters of the solution was distilled. When the solution was cool, an excess of ether was added, and the solution was washed successively with water, with 5% sodium bicarbonate (until the washings were neutral), and with saturated sodium chloride, and then dried. Removal of the ether under reduced pressure gave an oil, which was dissolved in hexane and

chromatographed on alumina (15 g). Elution with benzene–hexane (1:1) gave 4β-acetoxy-5α-cholestan-5-ol (IX) as a crystalline solid. Crystallization from methanol gave plates, m.p. 177–178°. Reported m.p. 175–176°, [α] +38° (25). This diol monoacetate was converted to 4β-acetoxy-cholest-5-ene (X*c*) by a published method (reported m.p. 108°, [α] −70° (25)). After chromatography and crystallization from acetone–methanol, we obtained X*c* with m.p. 111.5–112°, [α] −71° (20–25% overall yield from cholest-4-ene).

Photosensitized Oxygenation of Cholest-4-ene (I)

Exploratory oxygenations revealed that conversion was largely complete after 24 h. Longer reaction (e.g. 48 h, 72 h) led to diminution of hydroperoxidic infrared bands and gradual increase in the intensities of bands attributable to ketonic breakdown products (e.g. 1 710, 1 660 cm^{-1}); consequently, shorter reaction times were chosen for the definitive runs described below.

Run 1

A solution of cholest-4-ene (1.00 g, m.p. 77–79°) and hematoporphyrin (0.008 g) in pyridine (75 ml) was oxygenated and irradiated for 25 h. After normal work-up, the product contained 6% α,β-unsaturated ketone (ultraviolet intensity at 240 mμ), and quantitative hydrogenation of an aliquot indicated a hydroperoxide content of 74% (based on a molecular weight of 402). The specific rotation (+28°) of another aliquot in chloroform gradually increased with time and reached +64° after 48 h, with no further change after an additional 12 h. A portion of the original crude product (0.70 g) was reduced with sodium iodide and the resulting oil ([α] +48°) was dissolved in hexane and chromatographed on alumina (25 g). Elution with benzene–hexane (1:10) gave a clear oil (0.114 g) whose infrared spectrum indicated it was largely (at least two-thirds) cholest-4-ene, with some contamination by unidentified material having bands at 1 095, 1 080, 895, and 710 cm^{-1}.

Elution with benzene–hexane (1:4) gave crystalline fractions (0.167 g, m.p. 65–74°) shown to be 5α-cholest-3-en-5-ol (VIII*a*) from the infrared spectra. Crystallization from methanol gave m.p. 72–74° ([α] −15°) which was not depressed by VIII*a* (m.p. 74–75°) prepared as described earlier. The analytical sample (m.p. 75–76°) was obtained by rechromatography on alumina and elution with hexane. Thin-layer chromatography on silica gel gave only one spot.

Anal. Calcd. for $C_{27}H_{46}O$ (386.64): C, 83.87; H, 11.99. Found: C, 83.87; H, 11.74.

Benzene–hexane (1:2) eluted a pasty solid (0.068 g) whose infrared spectrum indicated it was a mixture of VIII*a* and cholest-5-en-4β-ol (X*a*), roughly in the ratio 1:4, along with a small amount of ketonic material. A separate photooxygenation (see run 3) was conducted to isolate and identify X*a*. Continued elution gave fractions (0.014 g) identified as 5β-cholest-3-en-5-ol (VI*a*) by infrared comparison with authentic material, followed by fractions of a clear oil (0.231 g), which was largely cholest-4-en-3α-ol (V*a*) according to its infrared spectrum. For confirmation, a portion of the clear oil was acetylated and the acetate was crystallized from acetone–methanol, m.p. 82–83°, [α] +170°. The melting point was not depressed by the authentic acetate V*c*. A second portion of the clear oil was treated with Girard's reagent (27) to remove ketonic impurity and, after chromatography on alumina, gave V*a* whose infrared spectrum was the same as that of authentic material.

Methylene chloride containing increasing proportions of acetone eluted crude crystalline cholest-4-en-3β-ol (IV*a*, 0.046 g, m.p. 113–120°) identified by infrared comparison. A portion was benzoylated and gave, after crystallization from methanol, pure cholest-4-en-3β-ol benzoate identical in all respects (melting point, mixture melting point, and infrared spectrum) with an authentic sample.

Final elution with acetone gave unidentified oily mixtures (0.024 g) containing hydroxyl and carbonyl absorption in their infrared spectra. The total recovery from the column was 0.664 g (95%). Cholest-4-en-3-one (III) was not separately isolated in this run but its presence in several of the fractions was evident from the infrared spectra.

Run 2

This photooxygenation of cholest-4-ene (0.880 g) and hematoporphyrin (0.008 g) in pyridine (75 ml) was conducted for 24 h in a cold room that maintained the solution at about 15°. The crude hydroperoxidic product had an infrared spectrum similar to those from the other runs except that carbonyl absorption was absent, an indication that the hydroperoxides underwent less breakdown at the lower temperature. However, when the product was reduced with sodium iodide, appreciable cholest-4-en-3-one was produced (ultraviolet spectrum). We believe that the inadvertent use of too much acetic acid in the reduction promoted decomposition of the hydroperoxides. Chromatography gave the same products as in run 1. In addition, cholest-4-en-3-one (III) was identified (by infrared and ultraviolet spectra) in several of the fractions eluted with benzene–hexane (1:2).

Run 3

*Identification of cholest-5-en-4β-ol (X*a*).*—Cholest-4-ene (0.350 g) was photooxygenated for 24 h as in run 1. The product was reduced with sodium iodide and then treated with Girard's reagent (27) to remove ketonic material. Chromatography on alumina gave, in some of the fractions eluted with benzene–hexane, a white solid (0.027 g) whose infrared spectrum corresponded to that of authentic X*a*. Acetylation gave cholest-5-en-4β-ol acetate (X*c*), with m.p. 108° after recrystallization from acetone–methanol. Mixture melting point and infrared determinations established its identity with authentic X*c*.

Photooxygenation and rearrangement of hydroperoxides.—A solution of cholest-4-ene (1.0 g) and hematoporphyrin (0.006 g) in pyridine was irradiated and oxygenated for 24 h. The crude product (0.950 g), which contained ca. 6% of enone (ultraviolet analysis), was dissolved in chloroform (100 ml) and allowed to stand in the dark at room temperature until the optical rotation attained a constant value (48 h). The chloroform was removed with a rotary evaporator and the residue was reduced with sodium iodide.

A portion of the reduced product (0.350 g) was treated with Girard's reagent (27) to remove the ketonic component, and the non-ketonic material was chromatographed on alumina (9 g). All fractions that contained cholest-4-en-3α-ol (V*a*) and cholest-4-en-3β-ol (IV*a*) were combined (0.170 g, eluted with benzene–hexane from 1:4 to 1:1). Infrared inspection showed that the 3β-alcohol IV*a* was only a minor component of the mixture. Neither of the cholest-3-en-5-ols (VI*a* or VIII*a*) was detected during the chromatography.

A second portion of the reduced product was acetylated and 0.242 g of the derived acetate was chromatographed on alumina (12 g). Elution with petroleum ether gave 0.180 g of fractions containing only the two cholest-4-en-3-ol acetates V*c* and IV*c*. Semiquantitative assay of the epimeric ratio was obtained by comparison of the infrared spectrum with authentic mixtures of the two acetates and indicated a V*c*:IV*c* ratio greater than 8:1. No 5α- or 5β-cholestan-3-en-5-ols were detected in the chromatography.

Attempted photooxygenations without sensitizer.—(A) A solution of cholest-4-ene (0.450 g, m.p. 80.5–81.5°) in pyridine (35 ml) was irradiated and oxygenated for 26 h in the absence of sensitizer. The olefin (m.p. 79–80°) was recovered virtually quantitatively and its identity with starting material was confirmed by melting point, mixture melting point, and infrared inspection. (B) A solution of cholest-4-ene (202 mg, containing 1.6% of cholesta-3,5-diene, based on ultraviolet absorption in isooctane at 228, 235, and 244 mμ (28)) in pyridine (15 ml) was photooxygenated 25 h without sensitizer. During this period a solution of 0.9 equivalent of *t*-butyl hydroperoxide in pyridine (1 ml) was added portionwise (0.1 ml initially and every 2 hours, the remainder after 8 h). Normal work-up left a solid (quantitative yield), which gave a positive hydroperoxide test and whose infrared spectrum showed very weak absorption at 3 540 and 1 680 cm^{-1}, but which otherwise appeared identical with that of starting material. The ultraviolet absorption (isooctane) at 232 mμ indicated ca. 3.5% cholest-4-en-3-one. The product was allowed to stand for 2 days in chloroform and then was reduced with potassium iodide in the usual way. Finally, it was oxidized with manganese dioxide, after which the enone content (λ 232) was 4.3–6.8%. The range arises because of the diene, whose presence was indicated by an inflection at 244 mμ but whose contribution to the 232-mμ peak cannot be accurately evaluated.

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