Sodium Borohydride Reduction of Steroidal Ketones with and without Irradiation

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Abstract: The sodium borohydride reduction of cholestan-3-one in 2-propanol was not accelerated by ultraviolet irradiation. However, the rate of the photoreduction of 4-cholesten-3-one (I) was enhanced by a factor of 22.5. The photoreduction of I with sodium borohydride gave 4-cholesten-3β-ol (II, 34%), 5α-cholestan-3β-ol (III, 16%), 5α-cholestan-3α-ol (IV, 8%), cholestane-3β,5α-diol (V, 6%), cholestane-3α,5β-diol (VI, 6%), an unknown sterol (VII, 7%), an (olefinic) hydrocarbon (VIII, 5%), and starting material (3%). This reaction was also studied in the presence of naphthalene, 1,3-pentadiene, and oxygen. The ground-state reduction of 4-cholesten-3-one (I) gave II, III, IV, an unknown sterol IX, and a (saturated) hydrocarbon X. The mechanism of these reactions is discussed.

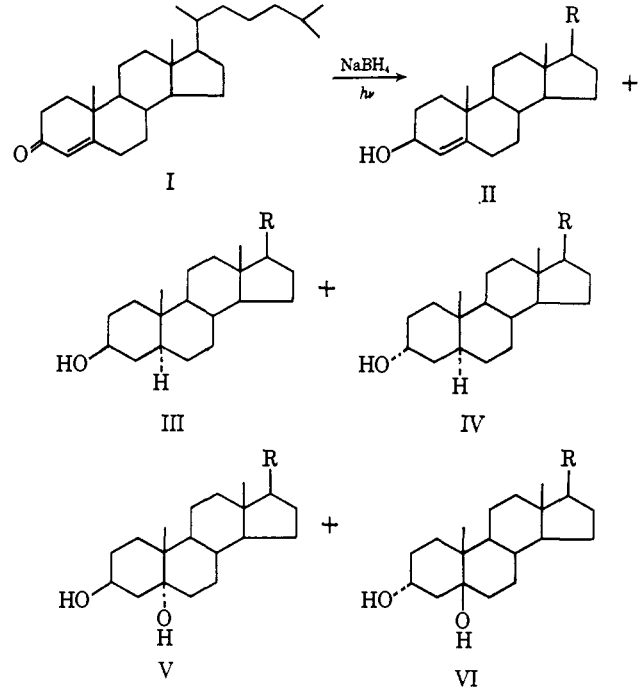
The phenolic part of 3,17β-estradiol has been photoreduced to the tetrahydro and hexahydro derivatives in the presence of sodium borohydride and inorganic sulfites.[1] This investigation has now been extended to unsaturated carbonyl systems. This paper describes the influence of ultraviolet irradiation on the borohydride reduction of steroidal saturated and α,β-unsaturated ketones. Changes in the reduction rates, stereochemical alterations, and the general composition of reaction mixtures in the reduction of cholestan-3-one and 4-cholesten-3-one were determined. The photoreductions of these steroidal ketones were compared with borohydride reductions in the ground state and with the effects of irradiations in the absence of reducing agent.

Methods and Results

A solution of cholestan-3-one in 2-propanol containing a 16 *M* excess of sodium borohydride was irradiated at 36° with a 450-w Hanovia medium-pressure mercury lamp equipped with a Pyrex filter. Aliquots of the solution were removed periodically from 0 to 10 min. The reaction was quenched by pouring the aliquots into water and by extraction with ether. Each extract was subjected to glpc. The relation of unreacted ketone to reduced product was determined by comparison of the peak areas. Tlc resolved the reaction mixture (10 min) into two reduction products, 5α-cholestan-3β-ol and 5α-cholestan-3α-ol.[2] Control reductions without irradiation gave comparable results. Therefore, in the case of a saturated steroidal ketone, such as cholestan-3-one, irradiation has no effect on the rate of borohydride reduction or the composition of the products. In addition, cholestan-3-one was unchanged after photolysis for 1 hr in the absence of sodium borohydride.

The rate of reduction of 4-cholesten-3-one (I) was easily followed by the disappearance of the ultraviolet absorption band at 240 mμ. In the ground state at 36°, in a given experiment, 90% of I was reduced in 270 min, as calculated from optical density measurements. Tlc resolved the reaction mixture into five compounds (see below). Irradiation enhanced the rate of borohydride reduction of I by a factor of 22.5 with 90% of the reaction complete within 12 min. Eight compounds were present in this reaction mixture as indicated by tlc. Irradiation of 4-cholesten-3-one (I) alone in 2-propanol gave a complex mixture of 14 products. The 240-mμ ultraviolet band disappeared (90%) in 59 min. The major irradiation product had a tlc R_f value comparable to a saturated ketone, *e.g.*, cholestan-3-one, and was presumably *lumicholestenone*,[3] which has been obtained by irradiation of 4-cholesten-3-one in *t*-butyl alcohol. Since the products of irradiation were not isolated, the extent to which this reaction is competing with photoreduction is not known. However, reduction products derived from *lumicholestenone* were not observed in the photoreduction.

The following products of the photoreduction of 4-cholesten-3-one (I) carried out on a preparative scale were isolated by column and preparative thin layer chromatography:[4] 4-cholesten-3β-ol (II, 34%), 5α-



I II III IV V VI

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(2) O. H. Wheeler and J. L. Mateos, *Can. J. Chem.*, **36**, 1049 (1958).
(3) W. W. Kwie, B. A. Shoulders, and P. D. Gardner, *J. Am. Chem. Soc.*, **84**, 2268 (1962).

cholestan-3β-ol (III, 16%), 5α-cholestan-3α-ol (IV, 8%), cholestan-3β,5α-diol (V, 6%), cholestan-3α,5β-diol (VI, 6%), unknown sterol VII (7%), an unstable hydrocarbon VIII (5%), and starting material (3%).

The diol V was obtained as colorless needles, mp 223–226°, $[\alpha]^{20}D$ +17.9° (lit.[5] mp 223–225°, $[\alpha]D$ +20°). The mass spectrum[6] showed no molecular ion (M^+) but displayed peaks at *m/e* 386 ($M^+ - 18$, H_2O), 371 ($M^+ - 18 - 15$, H_2O and CH_3), 368 ($M^+ - 36$, $2H_2O$), and typical fragmentations of ring D with loss of H_2O and $2H_2O$, *m/e* 231 and 213, and ring C fission with loss of H_2O and $2H_2O$ at *m/e* 163 and 145.

Acetylation of diol V with acetic anhydride–pyridine at room temperature afforded the 3-acetate, mp 187–188.5° (lit.[7] mp 183–185°), whose infrared spectrum displayed bands at 3605 (OH) and 1725 cm^{-1} (O-acetyl).

Cholestan-3α,5β-diol (VI) was obtained as colorless needles, mp 193.5–195°, $[\alpha]^{20}D$ +32.9° (lit. mp 190–192.5°, $[\alpha]D$ +35°;[8] mp 192–193°, $[\alpha]^{22}D$ +47.1°[9]). The mass spectrum of VI displayed peaks similar to those of diol V. Cholestan-3α,5β-diol 3-acetate was obtained as flakes, mp 148.5–149.5° (lit.[9] mp 147–148°).

In addition to these compounds, an unidentified sterol VII, mp 118.5–120°, was obtained which gave an unusually high molecular ion, M^+ 512. This may be a dimeric product similar to the photoproduct[10] obtained by irradiation of 4-cholesten-3-one, in which cleavage at C-20,22 with subsequent dimerization was proposed.

Hydrocarbon VIII was obtained as a colorless solid, mp 56.5–59.5°, which was difficult to recrystallize and, judging from its behavior on tlc, readily underwent autoxidation. The infrared spectrum showed no hydroxyl absorption, and the nmr spectrum displayed olefinic proton absorption at δ 5.6.

Sodium borohydride reduction of I in the ground state afforded 4-cholesten-3β-ol (II; 44%), 5α-cholestan-3β-ol (III; 18%), and 5α-cholestan-3α-ol (IV; 12%). An unidentified (possibly not quite homogeneous) sterol, IX, mp 91.5–93° (7%), was also obtained. The R_f value (tlc) of IX was the same as that of 5β-cholestan-3β-ol (coprostan-3β-ol), mp 100–101°, and its mass spectrum displayed M^+ 388, *m/e* 370 ($M^+ - 18$, H_2O) and *m/e* 215 and 145, typical ring D and ring C fragmentations, respectively. An unstable, oily hydrocarbon, X, whose infrared spectrum showed no hydroxyl absorption, differed from the photoreduced hydrocarbon VIII by its nmr (no olefinic protons), infrared spectrum, and R_f value on tlc.

Discussion

4-Cholesten-3-one, though less sterically hindered than cholestan-3-one, is reduced in the ground state at a much slower rate due to the contribution of resonance structures which distribute the positive charge of the carbonyl group.[2] Formation of the olefinic alcohol II and of the saturated alcohols III and IV in the ground state can be explained on the basis of 1,2- and 1,4-hydride additions, respectively.[11] Several other instances have been recorded where sodium borohydride has reduced the double bond in steroidal[12] and other α,β-unsaturated ketones[13] to the corresponding alcohols.

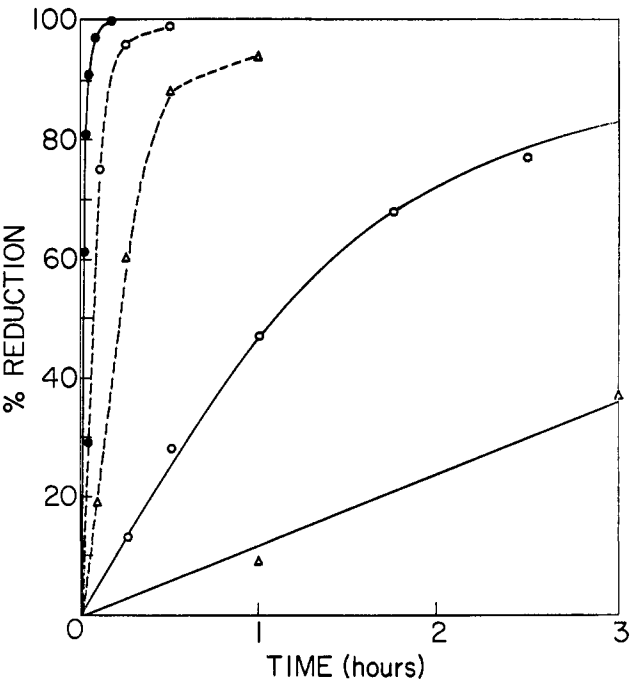

Figure 1. Relative rates of sodium borohydride reduction of 4-cholesten-3-one, O, and 1,4-cholestadien-3-one, △, in the ground state, ———, and under ultraviolet irradiation, - - - -. The rates for the reduction of cholestan-3-one, ●, in the ground and photoexcited states were identical.

The rate increase (22.5 times) in the photoreduction of 4-cholesten-3-one (I) with sodium borohydride in 2-propanol[14] (Figure 1) suggests the kind of intermediary photoexcited state operative in cyclic enone rearrangements.[15] On the other hand, the reducing agent may, under the influence of ultraviolet light, change to a radical species and become a donor of hydrogen atoms. In order to test this possibility we followed the reductive titer of borohydride solutions by the potassium iodate method.[16] Borohydride solutions are known not to reduce molecular oxygen.[17]

(4) The percentage yields of compounds designated throughout this paper are *estimated* yields based on tlc or glpc data of the various column and preparative tlc fractions obtained from the crude reaction mixture.

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(12) (a) J. A. Zderic and J. Iriarte, *J. Org. Chem.*, **27**, 1756 (1962); (b) D. Kupfer, *Tetrahedron*, **15**, 193 (1961); (c) F. Sondheimer and Y. Kibansky, *ibid.*, **5**, 15 (1959); (d) J. Fajkoš, *Collection Czech. Chem. Commun.*, **23**, 2155 (1958); (e) R. Albrecht and C. Tamm, *Helv. Chim. Acta*, **40**, 2216 (1957); (f) C. Djerassi, A. J. Manson, and H. Bendas, *Tetrahedron*, **1**, 22 (1957); (g) J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955); (h) F. Sondheimer, M. Velasco, E. Batres, and G. Rosenkranz, *Chem. Ind.* (London), 1482 (1954).

(13) (a) P. L. Southwick, N. Latif, B. M. Fitzgerald, and N. M. Zaczek, *J. Org. Chem.*, **31**, 1 (1966); (b) M. E. Cain, *J. Chem. Soc.*, 3532 (1964); (c) A. C. Currie, J. Gillon, G. I. Newbold, and F. S. Spring, *ibid.*, 773 (1960).

(14) 2-Propanol is an ideal solvent for kinetic studies of sodium borohydride; *cf.* H. C. Brown, O. H. Wheeler, and K. Ichikawa, *Tetrahedron*, **1**, 214 (1957); H. C. Brown and K. Ichikawa, *ibid.*, **1**, 221 (1957).

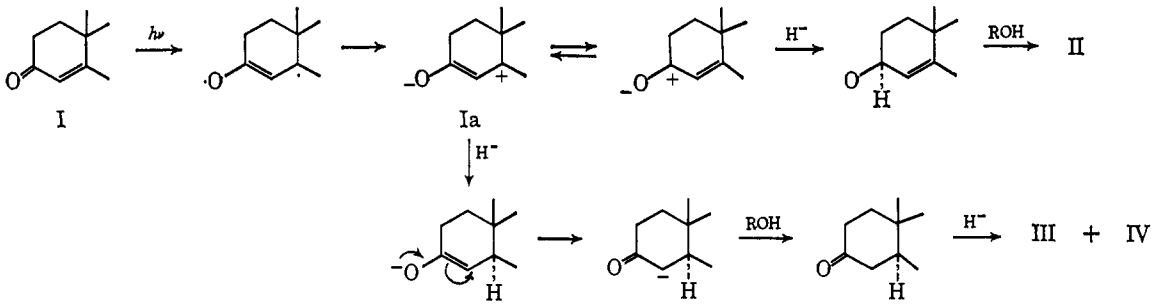
(15) (a) H. E. Zimmerman and D. J. Sam, *J. Am. Chem. Soc.*, **88**, 4905 (1966), and literature cited therein; (b) O. L. Chapman, *Advan. Photochem.*, **1**, 342 (1963); (c) N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, Inc., New York, N. Y., 1965, p 170.

(16) D. A. Lyttle, E. H. Jensen, and W. A. Struck, *Anal. Chem.*, **24**, 1843 (1952).

(17) *Cf.* R. E. Davis, E. Bromels, and C. L. Kibby, *J. Am. Chem. Soc.*, **84**, 885 (1962).

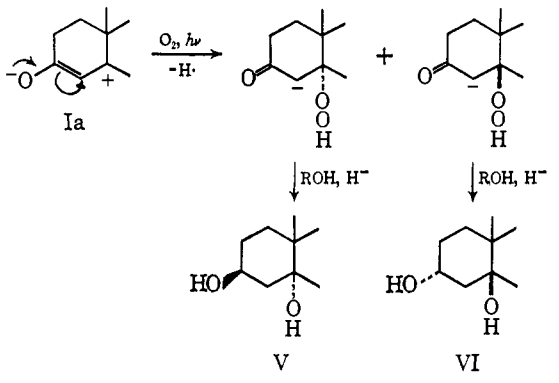
Should irradiation produce hydrogen atoms, such a reduction should take place. However, irradiation did not change the reduction titer of a borohydride solution through which a stream of oxygen was passed. In the light of this finding the zwitterionic intermediate[15] Ia is likely to be the species for hydride attack. Some reduction of the diradical intermediate by hydrogen abstracted from solvent cannot be discounted, however. There is no apparent change in rate or in the composition of products on irradiation of I with borohydride in the presence of 0.03 *M* piperylene or 0.01 *M* naphthalene, known triplet-state quenchers.

A plausible mechanism for the formation of II, III, and IV *via* zwitterion Ia is depicted as shown.



When the photoreduction of 4-cholesten-3-one was carried out in 2-propanol through which oxygen, a free-radical scavenger, was bubbled, 4-cholesten-3β-ol decreased and the HO-free hydrocarbon VIII increased. The diradical intermediate (triplet state *via* intersystem crossing from singlet biradical) might well be attacked with carbon–oxygen cleavage to give VIII, circumventing the formation of 4-cholesten-3β-ol (II). However, this argument is weakened by the observation that another hydrocarbon, X, is also formed in ground-state reduction.

Diols V and VI are formed from I by photoreduction only, and may arise *via* C-4,5 epoxides or C-5 hydroperoxides from small amounts of oxygen dissolved in 2-propanol. Photosensitized oxygenation of 4-cholesten-3β-ol (II) in pyridine with hematoporphyrin[18] gave 4α,5-epoxy-5α-cholestan-3-one and 4-cholesten-3-one. It was postulated that the epoxy ketone was formed *via* a 5-hydroperoxide intermediate. However, exposure of II to externally generated singlet oxygen did not convert II to an allylic hydroperoxide,[19] although other olefins were convertible to hydroperoxide.[19,20] Epoxides have been photolyzed to hydroxy compounds.[21,22]



Since hydroperoxides are often precursors of epoxides and are readily converted to hydroxy compounds, the initial step in the formation of V and VI might be the nonstereospecific addition of oxygen to the zwitterion (or diradical) of Ia.[23]

Products V and VI might also result from the nonstereospecific addition of water (present in 0.25% in 2-propanol) to C-5 of the photoexcited state with subsequent reduction of the 3-keto group. Such a photohydration would be analogous to the addition of methanol or water to the double bond of (+)-carene[24] and 1-menthene[25] and methanol to cyclohexene[26] to form methyl ethers or alcohols.

The photoreduction of cholestan-3-one was not faster than reduction in the ground state. This is not surprising in view of the fact that photochemical reactions of saturated cyclic ketones normally involve free-radical intermediates, initiated by n $\rightarrow$ π^* excitation of the carbonyl group. The reaction products then result from bond cleavage, hydrogen atom abstraction, and hydrogen transfer.[27]

A large rate enhancement (*ca.* 30) in the photoreduction of 1,4-cholestadien-3-one with borohydride (Figure 1) was observed in preliminary experiments. Further work on this reaction is now in progress.

Experimental Section

Materials Used. 2-Propanol (Fisher, Spectroanalyzed grade) containing 0.25% water was used as solvent. Commercial grades of sodium borohydride, naphthalene, and 1,3-pentadiene (piperylene) were used without further purification. Cholestan-3-one (Southeastern Biochemicals) and 4-cholesten-3-one (Mann Laboratories) were checked for purity by tlc and/or glpc prior to photolysis. Silica gel G tlc plates (0.25 mm thick) were employed in all cases.

Apparatus. Melting points were made on a Kofler hot stage and are uncorrected. Optical rotations were measured in chloroform. The glpc data were obtained on a Barber-Colman series 5000 gas chromatograph using a 1% QF-1 on a column of Gaschrome P (80–100 mesh) at a temperature of 220°, 30 psi. The ultraviolet spectra were recorded in 2-propanol on a Beckman DB-G grating spectrophotometer. The infrared spectra were measured on a Perkin-Elmer spectrometer (Model 21) in chloroform, the nmr spectra on a Varian A-60 spectrometer in deuteriochloroform with tetramethylsilane as internal standard, and the mass spectra on the mass spectrometer AEI MS 9 at 70 ev. The irradiations were performed with a 450-w Hanovia mercury vapor lamp, 679A-36, using a water-cooled quartz immersion well

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(27) R. Srinivasan, *Advan. Photochem.*, **1**, 83 (1963).

equipped with a Pyrex filter. Unless stated otherwise nitrogen was passed through the solution during the entire irradiation period.

Sodium Borohydride Reduction of Cholestan-3-one. A. Ground State. A solution of 173 mg (0.45 mmole) of cholesten-3-one in 50 ml of 2-propanol, warmed to 36°, was added to 272 mg (7.2 mmoles) of sodium borohydride in 100 ml of 2-propanol (36°). The resulting solution (zero time on admixture) was placed in a constant temperature bath (36°). Aliquots of 5 ml were removed at 0, 1, 2, 3, 4, 5, and 10 min. Each aliquot was immediately poured into a separatory funnel containing 10 ml of water to quench the reaction. The mixture was extracted with 5 ml of ether, dried over sodium sulfate, and evaporated. A glpc chromatogram of each aliquot gave the ratio of cholestan-3-one to reduced cholestan-3-one by integration of the peak areas (see Table I). The glpc showed only a single peak for reduced cholestan-3-one. However, tlc in two solvent systems (dichloromethane–acetone, 92:8, and hexane–acetone–ether, 8:1:1) showed the presence of 5α-cholestan-3β-ol as the major product and 5α-cholestan-3α-ol as a minor component.

Table I

Time, min	Cholestan-3-one, %	Reduced cholestan-3-one, %
0	100	0
1	42	58
2	19	81
3	9	91
4	8	92
5	3	97
10	0	100

B. Photoreduction of Cholestan-3-one. In *all* irradiation experiments the Hanovia lamp was turned on 10 min before the addition of the solutions to the photolysis chamber. This time period was required for 2-propanol solutions to reach and maintain a constant temperature of 36°. Solutions of cholestan-3-one and sodium borohydride were prepared in identical fashion as described above. After warming each to 36°, the solutions were mixed and immediately placed in the photolysis cell. Aliquots were removed, quenched, and extracted, and the glpc data recorded (see Table II). The reduced cholestan-3-one was resolved by the two tlc systems described above into 5α-cholestan-3β-ol, the major product, and 5α-cholestan-3α-ol, the minor product.

Table II

Time, min	Cholestan-3-one, %	Reduced cholestan-3-one, %
0	100	0
1	39	61
2	25	75
3	13	87
4	9	91
5	4	96
10	0	100

Irradiation of Cholestan-3-one. As a control experiment, 173 mg of cholestan-3-one in 150 ml of 2-propanol, in the absence of borohydride, was irradiated as described above. Aliquots were removed at 0, 15, 30, 60, 120, and 240 min and examined by tlc (hexane–acetone–ether, 8:1:1). Aliquots at 60 min and earlier showed no disappearance of starting material. Only after 120 and 240 min did trace amounts of new products begin to appear on the chromatogram.

Sodium Borohydride Reduction of 4-Cholesten-3-one. A. Ground State. A solution of 173 mg (0.45 mmole) of 4-cholesten-3-one in 50 ml of 2-propanol (36°) was added to a solution of 272 mg (7.2 mmoles) of sodium borohydride in 100 ml of 2-propanol (36°). The resulting solution was placed in a constant temperature bath (36°), with nitrogen bubbled through the solution. The disappearance of the α,β-unsaturated ketone absorption in the ultraviolet was recorded in units of optical density (0.2-ml aliquots diluted to 10 ml of 2-propanol). The experiment was repeated to obtain duplicate values for each period (see Table III). On completion of the 390-min reaction period, the solution was diluted with 300 ml of water, extracted with ether, dried, and evaporated. Tlc (hexane–acetone–ether, 8:1:1) of the material showed the presence of five compounds whose isolation will be described in the preparative scale experiment.

Table III

Time, min	$OD_{240m\mu}$	Time, min	$OD_{240m\mu}$
0	0.90, 0.95	105	0.28, 0.30
6	0.81, 0.86	150	0.23, 0.20
15	0.80, 0.80	210	0.13, 0.12
30	0.65, 0.67	300	0.07, 0.05
69	0.46, 0.52	390	0.02, 0.02

B. Photoreduction of 4-Cholesten-3-one. Solutions of borohydride and 4-cholesten-3-one were prepared as described above, mixed, and irradiated. Aliquots were removed periodically and the ultraviolet absorption bands at 240 mμ recorded [time, min ($OD_{240m\mu}$)]: 0 (0.86, 0.84), 2 (0.56, 0.64), 6 (0.21, 0.21), 15 (0.04, 0.03), and 30 (0.02, 0.00). The reaction mixture was worked up in the usual manner. Tlc indicated the presence of seven major compounds. Comparison by tlc of this mixture and the products from the control experiment to be described suggested the absence of *lumicholestenone*[3] or borohydride-reduced *lumicholestenone*. The isolation of these compounds is described in the preparative scale experiment.

Addition of Naphthalene. To a solution of 4-cholesten-3-one and sodium borohydride (the same concentrations as above) was added 192 mg (0.01 *M*) of naphthalene. The solution was irradiated for the normal period of 30 min. Comparison of this reaction mixture with that of the photoreduced 4-cholesten-3-one by tlc in three solvent systems (dichloromethane–acetone, 92:8; hexane–acetone–ether, 8:1:1; hexane–ethyl acetate, 7:3) showed no significant quenching of any of the products, their ratios, or a change in the rate of reduction.

Addition of Piperylene. Piperylene (1,3-pentadiene, 306 mg, 0.03 *M*) was added to the usual 4-cholesten-3-one and sodium borohydride solution (150 ml) and irradiated for 30 min. Inspection of the reaction mixture on tlc (three solvent systems) as described above suggested no significant quenching of the reaction products.

Presence of Oxygen. A solution of 173 mg (0.45 mmole) of 4-cholesten-3-one in 50 ml of 2-propanol (36°) was added to a solution of 272 mg (7.2 mmole) of sodium borohydride in 100 ml of 2-propanol (36°) through which oxygen was bubbled first for 2–3 min, and then for the time of irradiation (30 min). To the solution was added 10 ml of 5% hydrochloric acid. Most of the solvent was evaporated under reduced pressure, followed by extraction with chloroform. The chloroform extract was washed with water, dried, and evaporated to give 178 mg of a colorless solid. Tlc of the material (three solvent systems) showed a great increase in the hydrocarbon compound (R_f 4.8), a diminished yield of 4-cholesten-3β-ol (both compounds described in the preparative scale experiment), and two products more than found in the photoreduction of 4-cholesten-3-one.

Irradiation of 4-Cholesten-3-one. In a control experiment, 173 mg of 4-cholesten-3-one in 150 ml of 2-propanol (36°) was irradiated (no borohydride) and ultraviolet spectra of the aliquots were taken [time, min ($OD_{240m\mu}$)]: 0 (0.95), 5 (0.82), 15 (0.45), 30 (0.20), and 60 (0.10). A tlc of the reaction mixture showed the presence of 14 products. A major product had an R_f value (tlc) comparable to that of a saturated ketone standard, *i.e.*, cholestan-3-one, and is most likely *lumicholestenone*.[3]

Preparative Scale Reduction of 4-Cholesten-3-one in the Ground State. A solution of 2.59 g (6.7 mmoles) of 4-cholesten-3-one in 2.25 l. of 2-propanol contained in a 3-l. erlenmeyer flask was warmed to 36°, and 4.08 g (108 mmoles) of sodium borohydride was added. The flask was placed in a constant temperature bath (36°) for 7 hr. The solution was diluted with 4.5 l. of water and extracted two times with 225-ml portions of chloroform. The combined extracts were washed four to five times with water and then dried over sodium sulfate. A duplicate experiment was carried out to give a combined yield of *ca.* 6.0 g of colorless solid. Tlc of the material showed the presence of five major compounds (*cf.* rate experiment). The crude product was subjected to column chroma-

Table IV

Fraction	Solvent, ml	Amount, g
g-1	Ether, 1200	0.388 oil
g-2	Methanol (0.1%) in ether, 2500	1.418 solid
g-3	Methanol (0.1%) in ether, 700	1.248 solid
g-4	Methanol (0.1%) in ether, 2500	1.644 solid
g-5	Methanol (0.5%) in ether, 1000	0.200 solid

tography (180 g of Woelm alumina, grade 1) which gave the fractions listed in Table IV.

Fraction g-1 (388 mg, 8% of total) showed a single spot on tlc (dichloromethane–ether, 92:8) with a high R_f value (0.76) indicative of a nonpolar substance. This material could not be crystallized and was therefore rechromatographed (20 g of alumina, grade 1). Elution with hexane gave 111 mg of product with no change in R_f value. Further elution with ether and methanol–ether mixtures gave 27 mg of polar material which remained at the origin on tlc. In a further attempt to obtain crystalline material, the 111-mg product was subjected to preparative tlc (hexane) to give only 18 mg of colorless oil (X), the remainder being more polar material. Diminishing yields on chromatography and failure to crystallize this compound suggest that it is undergoing spontaneous oxidation in air; infrared spectrum: no hydroxyl absorption; nmr spectrum: no olefinic protons.

Tlc of fraction g-2 (1.418 g) showed the presence of three compounds with R_f 0.51, 0.45, and 0.40. Column chromatography (47 g of alumina, grade 3) of the fraction, on elution with benzene–hexane (1:3 and 1:1), gave 857 mg of a mixture of the two higher R_f compounds. To obtain the R_f 0.51 compound (7% of total), the mixture was rechromatographed two more times on alumina (grade 3), the final procedure giving 52 mg of highly purified material on elution with benzene–hexane (1:9). Crystallization from methanol–acetone and then ethanol gave 7.8 mg of colorless solid, mp 182–186°. An additional recrystallization from ethanol–acetone raised the melting point to 188–189° (IV). A mixture melting point with 5α-cholestan-3α-ol (lit.[28] mp 187–188°) was 187.5–189° (no depression). The infrared spectrum and tlc R_f value of the sample were identical with the authentic compound.

Further elution of the above chromatogram (47-g column) with benzene–hexane (1:1, 300 ml) gave 82 mg of pure compound (R_f 0.45, 12% of total). Crystallization of this product from acetone gave 50 mg of colorless solid, mp 91–92°. Two additional recrystallizations from methanol gave small colorless clusters, mp 91.5–93°, $[\alpha]^{20}D$ +29.5° (IX). The R_f was identical with 5β-cholestan-3β-ol (coprostan-3β-ol) (lit.[29] mp 101–102°, $[\alpha]D$ +28°). The infrared spectrum showed minor deviations in the fingerprint region compared with the authentic standard; nmr spectrum: no olefinic protons; mass spectrum: M^+ 388, *m/e* 370, 215, and 145.

Elution of the above chromatogram (47-g column) with 300 ml of benzene gave 140 mg of highly purified compound (R_f 0.40, 44% of total). Crystallization of the product from acetone–dichloromethane gave 75 mg of 4-cholesten-3β-ol (II) as fine, colorless needles, mp 129–131°, $[\alpha]^{20}D$ +43.5°. Additional amounts of 4-cholesten-3β-ol were obtained by chromatography of fraction g-4 (1.644 g) on alumina (45 g, grade 3). Elution with benzene–hexane (1:1) gave 419 mg of colorless solid, which on crystallization from acetone–dichloromethane gave 325 mg of colorless needles, mp 131.5–132.5°, $[\alpha]^{20}D$ +45.7° (lit.[30] mp 130–132°, $[\alpha]D$ +46°).

4-Cholesten-3β-ol (26.5 mg) was acetylated with 0.2 ml of acetic anhydride in 1.0 ml of pyridine. After storage at room temperature overnight, the mixture was poured into 10 ml of cold 5% sodium carbonate solution. The precipitated oil, after trituration with a glass rod for 10 min, crystallized. The crystals were collected, dried, and recrystallized from methanol to give 21.7 mg of needles, mp 85.5–86.5°, $[\alpha]^{20}D$ +12.7° (lit.[30] for 4-cholesten-3β-ol acetate, mp 87–88°, $[\alpha]D$ +10°).

In addition to 4-cholesten-3β-ol, fraction g-4 contained another compound, R_f 0.34, 18% of total crude product. Subsequent fractions eluted from the g-4 column with benzene–hexane (1:1) gave 156 mg of colorless solid which was according to tlc 85% pure. The product was crystallized from methanol–dichloromethane to give 114 mg of colorless flakes, mp 141.5–142°, inhomogeneous by tlc. Rechromatography on alumina gave 29 mg of pure material, recrystallized from methanol–dichloromethane and then dilute ethanol to give 18.8 mg of colorless flakes (III), mp 140.5–141°. The sample was identified with 5α-cholestan-3β-ol (lit.[31] mp 141–142°) by mixture melting point, infrared spectra, and tlc.

Anal. Calcd for $C_{27}H_{48}O$: C, 83.43; H, 12.45. Found: C, 83.18; H, 12.41.

The acetate of 5α-cholestan-3β-ol (36.2 mg of crude product) was prepared with acetic anhydride in pyridine. Chromatography of the crude acetate (grade 3 alumina, elution with hexane) gave a product which was recrystallized four times from methanol–ethyl acetate to give colorless scales, mp 107–107.5° (lit.[31] mp 108–109°).

Certain chromatographic fractions were rechromatographed to determine if the epimeric 4-cholesten-3α-ol was present. This compound, mp 84°, was not obtained.

Preparative Photoreduction of 4-Cholesten-3-one. A solution of 1.152 g (3 mmoles) of 4-cholesten-3-one in 300 ml of 2-propanol (36°) was added to a solution of 1.8 g (48 mmoles) of sodium borohydride in 700 ml of 2-propanol (36°). The solution was added to the irradiation chamber. Aliquots were removed periodically and ultraviolet absorption spectra determined (25 min, $OD_{240m\mu}$ 0.04). After the 30-min irradiation period, the reaction mixture was poured into 2 l. of water and extracted two times with 150-ml portions of chloroform, and the extracts were dried over sodium sulfate. Evaporation of the solvent gave 1.33 g of crude white solid. The above reaction was repeated nine times to give a combined yield of 12.8 g. Tlc of the crude material indicated the presence of eight major compounds. The crude product was subjected to column chromatography on alumina (Woelm, grade 1, 600 g). The fractions obtained are listed in Table V.

Table V

Fraction	Solvent	Amount, g
1	Benzene–hexane (1:9, 2 l.)	0.07 oil
2	Benzene–hexane (1:3) to ether (14 l.)	0.63 solid
3	Methanol (1%) in ether (3 l.)	7.63 solid
4	Methanol (2.5%) in ether (2 l.)	1.08 solid
5	Methanol (5%) in ether (2 l.)	0.85 solid
6	Methanol (10 and 25%) in ether and chloroform–ethyl acetate–methanol (1:1:1, 4 l.)	0.66 solid

Fraction 2 (0.63 g, 5%) contained a nonpolar compound to judge by the high R_f value of 0.48 (tlc, hexane) in addition to trace amouts of impurities. This material was rechromatographed on alumina (40 g, grade 1). Elution with hexane gave 336 mg of a colorless oil which crystallized on standing. Recrystallization of the solid from ether–acetone gave 272 mg of colorless needles, mp 50–60°. A second recrystallization from the same solvent raised the melting point of the compound to 58–62°; *ca.* 10% impure by tlc. Preparative tlc (hexane) of the material and elution of a portion of the blue zone shown on the tlc plate under ultraviolet light gave 39 mg of product. Recrystallization from ether–ethanol gave 18.8 mg of crystalline solid, mp 56.5–59.5° (VIII). Approximately 60% of the original crystalline material stayed at the origin of the tlc plate, suggestive of rapid autoxidation. While the crystalline material is relatively stable in air as shown by tlc, a solution of the compound, allowed to evaporate to a thin film and left standing for several days, is almost completely oxidized. The crystalline compound (R_f 0.48) is not identical with the unstable oil (R_f 0.67) obtained in the ground-state experiment; infrared spectrum: no hydroxyl absorption, 1649 (w), 1592 (m), 1459 (s), 1432 (m), 1382 (s), 1369 (s), 1318 (m), 958 (w) cm^{-1}; nmr spectrum: δ 5.6 (multiplet presumably of olefinic protons).

Fraction 3 (7.63 g) contained five major compounds (R_f 0.52, 0.46, 0.43, 0.39, and 0.35) by tlc (hexane–acetone–ether, 8:1:1). The mixture was subjected to column chromatography (345 g of alumina, grade 1). Elution with 4.5 l. of 0.25% methanol in ether gave 6.29 g of a colorless solid containing the five compounds above, free of all impurities. Rechromatography of this material

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gave five fractions when progressively eluted with 0.25% methanol in ether, designated as 3a, 1.44 g; 3b, 0.86 g; 3c, 0.18 g; 3d, 3.75 g; 3e, 0.57 g. Fraction 3a (compounds R_f 0.52 and 0.43) was rechromatographed two times on alumina, the final procedure (elution with ether–benzene, 1:9) giving 109 mg of impure colorless solid (R_f 0.43, 3% of total). Preparative tlc (hexane–acetone–ether, 9:0.5:0.5, developed continuously for 2 hr) of this material gave 80 mg of pure compound. Recrystallization from methanol furnished large colorless needles (I), mp 81.5–82.5°, $[\alpha]^{20}D$ +91.2°. The compound was identified as 4-cholesten-3-one by mixture melting point (80–81°), infrared, and tlc comparison with starting material.

Further elution of the 3a column with ether–benzene (1:1) gave 87 mg of a colorless solid (R_f 0.52, 7% of total). Two recrystallizations of the solid from dilute ethanol gave 15.8 mg of an unknown compound, VII, mp 118.5–120°. Analytical data as well as infrared and mass spectra gave inconclusive evidence as to the nature of this compound; infrared spectrum: 3600 (m), 1468 (s), 1382 (s), 1300 (s), 1168 (s), 1022 (m), 1008 (m) cm^{-1}; mass spectrum: M^+ 512.

Subsequent elution of the 3a column with 0.1% methanol in ether gave 835 mg of semisolid (two major compounds by tlc). This material was rechromatographed on alumina and then subjected to preparative tlc (hexane–acetone–ether, 9:0.5:0.5) developed continuously for 1.5 hr. Elution of a portion of the faint zone made visible on the plate by a short-wave ultraviolet lamp gave 220 mg of crystalline compound, enriched in the desired compound (R_f 0.46, 8% of total). Crystallization of the material from acetone–methanol and then ethanol gave 18 mg of colorless needles (IV), mp 184.5–186°, $[\alpha]^{20}D$ +17.3° (lit.[28] mp 187–188°, $[\alpha]D$ +24°). The compound was identical, by infrared spectra (superimposable) and tlc, with authentic 5α-cholestan-3α-ol obtained from the ground-state experiment.

Anal. Calcd for $C_{27}H_{48}O$: C, 83.43; H, 12.45. Found: C, 83.25; H, 12.44.

Fraction 3d (3.78 g) was chromatographed on alumina (175 g, grade 1). Elution with 0.5% methanol in ether gave 350 mg of solid (R_f 0.39, 34% of total). The product was recrystallized first from methanol and then from acetone–dichloromethane to give 218 mg of 4-cholesten-3β-ol (II) as needles, mp 129–130.5°, $[\alpha]^{20}D$ +40.9°, identical with the compound obtained from the ground-state reduction (mixture melting point, infrared spectra, tlc).

Further elution of the 3d column with 0.5% methanol in ether gave 961 mg of material enriched in the lower R_f compound (0.35, 16% of total). The mixture was rechromatographed two times and the purified material recrystallized first from acetone, then methanol–ether, and finally from dilute aqueous ethanol to give 5α-cholestan-3β-ol (III), mp 139–140°, identical with an authentic sample (mixture melting point, infrared spectra, tlc).

Fraction 5 (850 mg of yellow solid) contained two polar compounds (R_f 0.16 and 0.12) in *ca.* 1:1 ratio. Two recrystallizations from methanol–acetone gave 131 mg of pale yellow needles, mp 217–222°. Three additional recrystallizations from ethanol gave 5α-cholestan-3β,5-diol (V) as colorless needles, mp 223–226°, $[\alpha]^{20}D$ +17.9° (lit.[5] mp 223–225°, $[\alpha]^{20}D$ +20°); infrared spectrum: 3600 cm^{-1} (intense); mass spectrum: no M^+ ion, *m/e* 386, 371, 368, 231, 163, 159, and 145.

5α-Cholestan-3β,5-diol (V; 35 mg) was acetylated with 0.3 ml of acetic anhydride in 1.5 ml of pyridine (room temperature, 36 hr). Work-up of the mixture in the usual manner gave a solid product, which was recrystallized from methanol–ethyl acetate to give 29 mg of 5α-cholestan-3β,5-diol 3-acetate as pale yellow flakes, mp 187–188.5° (lit.[7] mp 183–185°); infrared spectrum: 3605 (OH) and 1725 cm^{-1} (O-acetyl).

The mother liquors from the fractional crystallizations were evaporated to give 265 mg of solid, which was recrystallized three times from ethanol to give pale yellow flakes, mp 195–197°, with transformation to needles. Two additional recrystallizations from ethanol gave 5β-cholestan-3α,5-diol (coprostan-3α,5-diol) (VI) as colorless needles, mp 193.5–195°, $[\alpha]^{20}D$ +32.9° (lit. mp 190–192.5°, $[\alpha]D$ +35°;[8] and mp 192–193°, $[\alpha]^{20}D$ +47.1°[9]); infrared spectrum: 3600 cm^{-1} (intense); mass spectrum: no M^+ ion, *m/e* 386, 371, 368, 231, 213, 163, 159, and 145.

The 3-monoacetate of 5β-cholestan-3α,5-diol (19 mg) was prepared with 0.4 ml of acetic anhydride in 1.7 ml of pyridine at room temperature for 36 hr. The solution was poured into 15 ml of cold sodium carbonate solution (5%). The solid was collected by filtration, dried, and recrystallized from ethyl acetate–methanol to give 12 mg of 5β-cholestan-3α,5-diol 3-acetate as pale yellow flakes, mp 148.5–149.5° (lit.[9] mp 147–148°); infrared spectrum: 3610 (OH) and 1730 cm^{-1} (O-acetyl).

Titrations of Sodium Borohydride in 2-Propanol. Four titration experiments were performed: (1) titration of the borohydride solution, (2) titration in the borohydride solution in the presence of oxygen, (3) titration of the irradiated borohydride solution, and (4) titration of the irradiated borohydride solution in the presence of oxygen. The solutions were prepared in the same manner. Sodium borohydride (170 mg) was dissolved in 150 ml of 2-propanol and placed in a constant temperature bath or irradiated (Pyrex filter). Aliquots (10.0 ml) of the 2-propanol solution were removed and titrated by the potassium iodate method.[16] To each aliquot was added 50.0 ml of 0.1 *N* potassium iodate containing 2 g of potassium iodide, followed by addition of 10 ml of 5.0 *N* sulfuric acid. To the mixture was added 2–3 ml of starch indicator solution and the solution was titrated with 0.1 *N* sodium thiosulfate. The volume of titrant and milligrams of borohydride at various time periods are summarized in Table VI.

Table VI

	Time, hr			
	0	1	2	4
(1) BH_4 solution				
Sodium thiosulfate, ml	31.7	31.5	32.0	...
Sodium borohydride, mg	8.66	8.75	8.51	...
(2) BH_4 solution + *hν*				
Sodium thiosulfate, ml	31.7	31.6	31.2	...
Sodium borohydride, mg	8.66	8.70	8.89	...
(3) BH_4 solution + O_2				
Sodium thiosulfate, ml	31.7	31.3	31.7	...
Sodium borohydride, mg	8.66	8.85	8.66	...
(4) BH_4 solution + O_2 + *hν*				
Sodium thiosulfate, ml	31.8	31.7	...	31.8
Sodium borohydride, mg	8.61	8.66	...	8.61

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