Irradiation Studies and Novel Sodium Borohydride Reduction of 1,4-Cholestadien-3-one

JAMES A. WATERS AND BERNHARD WITKOP

Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

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Irradiation of 1,4-cholestadien-3-one (I) in *t*-butyl alcohol gave four phenols, *i.e.*, 1-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene (II, 40%), 3-hydroxy-1-methyl-19-norcholesta-1,3,5(10)-triene (III, 40%), 4-hydroxy-2-methyl-19-norcholesta-1,3,5(10)-triene (IV, 10%), and 2-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene (V, 4%). Irradiation of dienone I in the presence of sodium borohydride afforded phenol II (15%), phenol III (45%), phenol IV (13%), 4-methyl-19-norcholesta-1,3,5(10)-triene (IX, 5%), and an unidentified sterol X (8%). Ground-state sodium borohydride reduction of dienone I gave 5β-cholest-1-en-3-one (VI, 10%), 4-cholesten-3-one (VII, 18%), 4-cholesten-3β-ol (VIII, 40%), hydrocarbon IX (8%), and starting material (12%). These reactions are discussed and compared with reductions of 4-cholesten-3-one reported previously.

The photoreduction of 3,17β-estradiol[1] and steroidal ketones[2] by ultraviolet irradiation in the presence of sodium borohydride has been described. A 22-fold rate increase in the photoreduction *vs.* ground-state reduction of 4-cholesten-3-one was observed. The products obtained by photo- and ground-state reduction in 2-propanol were essentially the same. We now describe the reactions of the cross-conjugated dienone 1,4-cholestadien-3-one (I), (i) under ultraviolet irradiation, (ii) with excess borohydride, and (iii) under irradiation in the presence of borohydride in *t*-butyl alcohol and 2-propanol. The unreactivity of ring-A steroidal dienones toward sodium borohydride coupled with the intriguing photochemical rearrangements of this system made this study especially desirable. In addition, the individual processes could be interrelated.

Methods and Results

Irradiations in the Absence of Borohydride.—A solution of 1,4-cholestadien-3-one (I, 0.7 mmol) in 150 ml of *t*-butyl alcohol was irradiated at 36° with a 450-W Hanovia medium-pressure mercury lamp (679A-36) equipped with a Pyrex filter. Aliquots of the solution were removed periodically from 0 to 60 min. The rate of photorearrangement of I was easily followed by the disappearance of the ultraviolet absorption band at 244 mμ; 90% of the reaction was complete within 29.5 min. A similar rate of rearrangement of I in 2-propanol was observed and tlc indicated the same composition of products.

The four compounds isolated from the preparative scale irradiation in *t*-butyl alcohol by column and preparative thin layer chromatography[3] were 1-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene[4-6] (II, 40%), 3-hydroxy-1-methyl-19-norcholesta-1,3,5(10)-triene[7] (III, 40%), 4-hydroxy-2-methyl-19-norcholesta-1,3,5(10)-triene (IV, 10%), and 2-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene[8] (V, 4%).

Phenol IV has been tentatively assigned the structure of 4-hydroxy-2-methyl-19-norcholesta-1,3,5(10)-triene, based on spectral data which is in excellent agreement with that of the analogous phenol, *i.e.*, 4-hydroxy-2-methyl-17β-acetoxyestra-1,3,5(10)-triene.[9]

Sodium Borohydride Reduction of Dienone I in the Ground State.—The kinetic data of the sodium borohydride reduction of dienone I are summarized in Table III (Experimental Section). No reduction took place at the stoichiometric concentration of sodium borohydride. At increasing concentrations, the rate of reduction was proportionately increased. Figure 1 illustrates the reduction of I at several concentrations of borohydride.

Reduction of dienone I on a preparative scale in *t*-butyl alcohol with an 8 molar excess of sodium borohydride gave the following four reduction products which were isolated by careful chromatography: 5β-cholest-1-en-3-one[10] (VI, 10%), 4-cholesten-3-one[11] (VII, 18%), 4-cholesten-3β-ol[11] (VIII, 40%), and 4-methyl-19-norcholesta-1,3,5(10)-triene[12] (IX, 8%) (Scheme I).

Irradiations of Dienone I in the Presence of Sodium Borohydride.—Dienone I was irradiated in the presence of an 8 molar excess of sodium borohydride. The time required for 90% disappearance of dienone I (uv) in *t*-butyl alcohol was 30 min, comparable with the reaction in 2-propanol. The preparative run gave five compounds: phenols II (15%), III (45%), IV (13%), hydrocarbon IX (5%), and an unknown sterol X (8%). Phenols II, III, and IV were identical with those obtained from the irradiation without borohydride by mixture melting point, infrared spectra, and tlc. The spectral properties (infrared, ultraviolet, and nmr) of 4-methylcholestatriene IX were comparable with the oily hydrocarbon obtained from the borohydride reduction. The unidentified sterol X which was obtained as a colorless solid, mp 112–113.5°, $[\alpha]^{20}D$ −45.3°, may be a reduced bicyclohexenone, an intermediate in the formation of phenolic isomers. The products and yields from the irradiation, borohydride reduction, and attempted photoreduction of dienone I are correlated in Table I.

(1) J. A. Waters and B. Witkop, *J. Amer. Chem. Soc.*, **89**, 1022 (1967).
(2) J. A. Waters and B. Witkop, *ibid.*, **90**, 758 (1968).
(3) The per cent yields of the various compounds reported in this paper are *estimated* yields from the total reaction mixture as determined by tlc and glpc of the fractions obtained from column and preparative thin layer experiments.
(4) A. L. Wilds and C. Djerassi, *J. Amer. Chem. Soc.*, **68**, 1712 (1946).
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TABLE I

IRRADIATION, BOROHYDRIDE REDUCTION, AND PHOTOREDUCTION OF CHOLESTADIENONE (I)

	Yields, %								
Dienone reaction	Phenol II	Phenol III	Phenol IV	Phenol V	Enone VI	Enone VII	Enol VIII	Hydrocarbon IX	Sterol X
$h\nu$	40	40	10	4					
$h\nu$ + $NaBH_4$	15	45	13					5	8
$NaBH_4$					10	18	40	8	

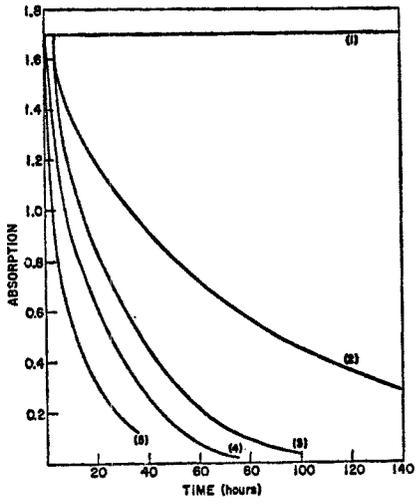
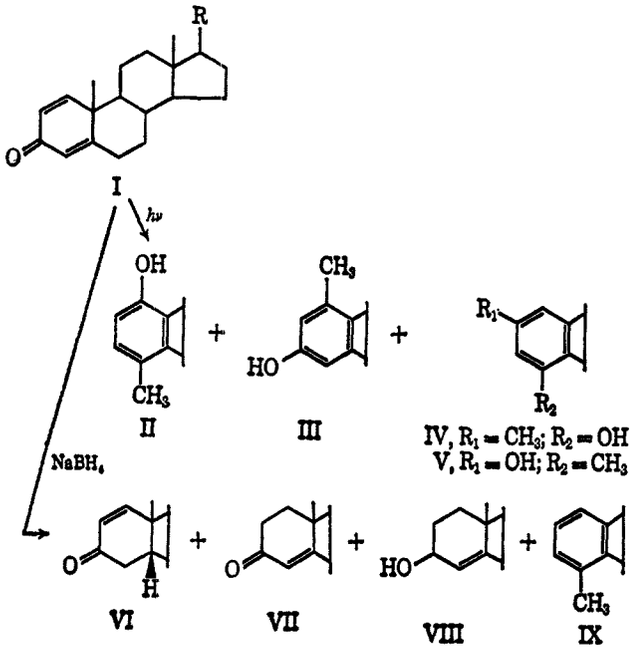


Figure 1.—Ultraviolet absorption of 1,4-cholestadien-3-one (0.023 mmol in 5.0 ml of *t*-butyl alcohol) at different $NaBH_4$ concentrations: (1) 0.006 mmol, (2) 0.031 mmol, (3) 0.056 mmol, (4) 0.12 mmol, and (5) 0.19 mmol.

SCHEME I



Discussion

Photorearrangement of 1,4-cholestadien-3-one (I)[13] to give phenols II–V parallels the irradiation of 1-dehydro-17-acetyltestosterone in neutral media[9,14,15] in which four phenols (9-hr reaction period) or four photoketones (1.5-hr reaction period) have been isolated. The cross-conjugated dienone, upon n–π^* excitation, is converted into a triplet species (singlet to triplet intersystem crossing), which is postulated to undergo bond alteration and formation of a mesoionic species.[16] This zwitterion may then rearrange to bicyclo[3.1.0]hexenones, which, in turn, undergo secondary photorearrangement to the spirocyclic 2,4-cyclohexadienones. The photoketones can then further rearrange to phenols of type II–V. Separate irradiation of each ketone has established this latter rearrangement.[9,15] The course of dienone rearrangement is greatly influenced by a 4-methyl substituent[9,15,17,18] or by the presence of acid.[15,17,18] Identical phenols may be formed by irradiation[19] or by the acid-catalyzed dienone–phenol rearrangement.[20]

To correlate photorearrangement with photoreduction, dienone I was reduced with sodium borohydride in the ground state. Figure 1 illustrates the kinetics of this reduction. At high borohydride concentration (0.187 mmol), 90% of the original dienone disappeared within 30 hr (extrapolation from Table III), whereas at low concentrations (0.006 mmol) no reduction took place, and in 0.03 mmol of $NaBH_4$ the reduction required 172 hr. Preparative-scale reduction at high borohydride concentration gave enones VI and VII, 4-cholesten-3β-ol (VIII), and hydrocarbon IX (Table I).

Steroidal ring-A dienones are usually resistant to reduction by sodium borohydride. Exceptions are the reductions of 1,4-androstadiene-3,17-dione[21] and prednisone[22] in which both the Δ^1 double bond and the carbonyl functions were reduced. Reduction of the Δ^4 double bond of I to give 5β-cholest-1-en-3-one (VI) and the Δ^1 double bond to 4-cholesten-3-one (VII) with preservation of the carbonyl group has no precedent. Reduction of both the 4,5 bond and the carbonyl group of a 1,4-dien-3-one with lithium aluminum hydride in boiling tetrahydrofuran has been noted.[23] Chemical[23] or enzymatic reduction of the 4,5 bond of the dienone[24] produced the 5β-1-en-3-one in analogy

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to our study in which 5β-cholest-1-en-3-one (VI, 1-coprosten-3-one) is formed.

4-Cholesten-3-one (VII) is reduced to 4-cholesten-3β-ol as described previously.[2] Hence VII is apparently formed to a much greater extent initially than the final yield indicates (Table I). Additional evidence that the Δ^1 double bond in 1,4-diene-3-keto steroids is more readily reduced than the Δ^4 double bond comes from the homogeneous catalytic hydrogenation technique.[25]

Formation of 4-methyl-19-norcholesta-1,3,5(10)-triene (IX) is analogous to the lithium aluminum hydride reduction of 1,4-dien-3-ones (to the 3ξ-ols) and subsequent rearrangement of the reaction mixture by alumina or Florisil chromatography.[23,26,27]

When I was irradiated in the presence of sodium borohydride, the dienone disappeared at the same rate as without borohydride. The products were predominantly phenolic (Table I). However, phenol II was formed in a much lower yield.

There is a striking contrast between this dienone photoreduction and the photoreduction of 4-cholesten-3-one[2] in which the same reduction products are formed as in the ground state, but at a rate increase of 22.5. The irradiation of dienone I in the presence of borohydride leads instead to phenolic rearrangement rather than reduction products. In the photoreduction of 4-cholesten-3-one in 2-propanol the solvent could be a source of hydrogen atoms. However, little or no difference in the rate of formation or composition of products was observed between *t*-butyl alcohol or 2-propanol, in contrast to the easy photoreduction of saturated 3-keto steroids in isopropyl alcohol solution.[28]

The rate and quantum efficiency of triplet rearrangement in dienones is up to 10,000-fold faster and 200-fold more efficient than in enones.[16,29] In addition, borohydride reduction of the dienone in the ground state is extremely slow. The intermediate zwitterionic bicyclic or spirocyclic photoproducts from the dienone[16] apparently rearrange to phenols fast enough that nucleophilic attack of hydride becomes negligible. Addition of a triplet quencher, such as 1,3-pentadiene,[28] should differentiate between these reactions by retarding the photorearrangements. Such attempted quenching experiments have so far been inconclusive, since interaction between borohydride and piperylene prevents attainment of desirable concentrations of quenching agent.

Experimental Section

Materials and Apparatus.—*t*-Butyl alcohol (Eastman Kodak) and 2-propanol (Fisher Spectroanalyzed grade) were used as solvents. Silica gel G tlc plates (0.25 mm thick) were used routinely and were developed in hexane–acetone–ether 8:1:1 unless designated otherwise. Melting points were taken on a Kofler hot stage and are corrected. Optical rotations were obtained on a Perkin-Elmer polarimeter (Model 141) in chloroform. The glpc data were obtained on a Barber–Coleman Series 5000 gas chromatograph using a 1% QF-1 column on Gaschrome P (80–100 mesh) at a temperature of 220° (30 psi). The ultraviolet spectra were recorded on a Beckman DB-G grating spectrophotometer in absolute ethanol unless stated otherwise. The infrared spectra were measured on a Perkin-Elmer spectrometer (Model 21) in chloroform, unless designated otherwise. The mass spectra were obtained on the LKB mass spectrometer and the nmr spectra on a Varian A-60 spectrometer in deuteriochloroform with tetramethylsilane as the internal standard. Samples were irradiated with a 450-W Hanovia mercury-vapor lamp, 679A-36, using a water-cooled quartz immersion well equipped with a Pyrex filter. Nitrogen was passed through the solution during the entire irradiation period.

1,4-Cholestadien-3-one (I).—Cholestan-3-one was converted into 2,4-dibromocholestan-3-one.[4] Dehydrobromination of the dibromo compound by redistilled collidine[30] gave 1,4-cholestadien-3-one, mp 113–114° (lit.[30] mp 111–111.5°). The compound was pure as indicated by tlc and glpc.

Irradiations of 1,4-Cholestadien-3-one. A. Kinetic Runs.—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 the alcohol solutions to reach and maintain a constant temperature of 36°. A solution of 267 mg (0.7 mmol) of 1,4-cholestadien-3-one in 150 ml of *t*-butyl alcohol was warmed to 36° and placed in the irradiation cell. Aliquots (0.2 ml diluted to 10 ml) were removed periodically and the ultraviolet absorption band at 244 mμ was recorded (time in minutes, OD units): 0, 1.70; 5, 1.25; 15, 0.51; 30, 0.16; 60, 0.12. On completion of the reaction period (75 min), the solvent was evaporated under reduced pressure. Tlc of the mixture showed the presence of four compounds. Glpc of the material displayed four peaks: 4.1 min (minor), 4.7 min (major), 4.9 min (minor), and 5.8 min (major). The two major compounds displayed a 1:1 peak area ratio. Isolation of these compounds will be described in the preparative scale experiment.

A solution of 154 mg (0.4 mmol) of 1,4-cholestadien-3-one in 150 ml of 2-propanol was irradiated and ultraviolet data were recorded in the manner described above (time in minutes, OD_{244}): 0, 1.20; 5, 1.05; 15, 0.58; 30, 0.22; 60, 0.07. Examination of the reaction mixture by tlc showed compounds with the same R_f value and intensity as the *t*-butyl alcohol irradiation. No products were isolated from the 2-propanol reaction.

B. Preparative Run.—A solution of 5.34 g (14 mmol) of 1,4-cholestadien-3-one in 3.0 l. of *t*-butyl alcohol was warmed to 36° and then placed in the photolysis chamber. The reaction was followed by noting the disappearance of the ultraviolet absorption as stated above (time in minutes, OD units): 0, 2.00; 20, 1.48; 80, 0.44; 120, 0.24; 150, 0.15. On completion of the irradiation period (180 min), the solvent was evaporated under reduced pressure. Tlc of the pale yellow semisolid showed the presence of four closely related compounds (R_f 0.42, 0.35, 0.32, 0.28). The crude mixture was subjected to column chromatography on alumina (Woelm, grade 3, 160 g). The following fractions were eluted as shown in Table II.

TABLE II
FRACTIONATION OF PHENOLIC REARRANGEMENT PRODUCTS FROM THE IRRADIATION OF DIENONE I

Fraction	Solvent (vol)	Material, g
1	Hexane (1 l.)	Solid (II), 1.04
2	Benzene–hexane 1:9 (1 l.)	Solid (II), 1.30
3	Benzene–hexane 1:3 (1 l.)	Oil (IV), 0.46
4	Benzene–hexane 1:1 (1 l.)	Oil (V), 0.65
5	Benzene (1 l.)	Oil (III), 1.81
6	Benzene–ether and ether–methanol mixtures (9 l. total)	Oil, 0.22

Fractions 1 and 2 contained the compound with R_f 0.42, 40% of total reaction mixture, in highly purified form as shown by tlc. Fraction 2 (1.3 g) was recrystallized from petroleum ether (bp 30–60°) to give 595 mg of colorless needles, mp 144.5–145.5°. The mother liquor, on standing, yielded a second crop, 171 mg, mp 145–146°. The combined material was recrystallized a

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TABLE III

KINETIC STUDY OF THE REDUCTION OF CHOLESTADIENONE WITH SODIUM BOROHYDRIDE

Tube no.[a]	$NaBH_4$, mg (mmol)	OD Time, hr 0	4	8	12	24	36	48	76	100	150	192
1	0.22 (0.0058)	1.70	1.70	1.70	1.70	1.70		1.70	1.70	1.70	1.70	1.70
2	1.17 (0.031)	1.70	1.70	1.38	1.35	1.16		0.80	0.62	0.43	0.25	0.10
3	2.10 (0.0556)	1.70	1.70	1.20	1.08	0.74		0.39	0.09	0.03		
4	3.07 (0.081)	1.70	1.60	1.20	0.87	0.57		0.28	0.09	0.02		
5	4.00 (0.106)	1.70	1.25	0.97	0.76	0.49	0.31	0.16	0.02	0.02		
6	4.93 (0.130)	1.70	1.36	0.82	0.67	0.35	0.26	0.06				
7	5.90 (0.156)	1.70	1.30	0.86	0.58	0.31	0.17	0.04				
8	7.07 (0.187)	1.70	1.04	0.74	0.49	0.22	0.12	0.01				

[a] Each tube contained 8.9 mg (0.023 mmol) of 1,4-cholestadien-3-one made up to a final volume of 5.0 ml of *t*-butyl alcohol.

second time from the same solvent to yield 532 mg of 1-hydroxy-4-methyl-19-norcholesta-1,3,5-(10)-triene (II) as colorless needles, mp 146.5–147.5°, $[\alpha]^{20}D$ +158.4°. An analytical sample was recrystallized a third time and gave mp 147.5–148° (lit. mp 145.5–146°, $[\alpha]^{24.5}D$ +161°[4,6] and mp 145–146°[5]); ultraviolet spectrum λ_{max} 284 mμ (ε 2400), 226 (5200); infrared spectrum 3595, 1587, 1480, 1465, 1382, 1300, 1155 cm^{-1}; in CS_2 900, 850, 810, 796, and 733 cm^{-1}; nmr spectrum (δ) 2.28 (s, aromatic CH_3), 6.97 (m, 2-CH and 3-CH).

Anal. Calcd for $C_{27}H_{42}O$: C, 84.75; H, 11.07. Found: C, 84.41; H, 10.81.

Additional amounts of phenol II, in less pure form, were obtained by recrystallization of fraction 1 from petroleum ether (bp 30–60°).

Fraction 3 (0.46 g) was highly enriched in compound R_f 0.35 (10% of total reaction mixture), but attempts to crystallize this compound from a variety of solvents was unsuccessful. The material was therefore chromatographed on alumina (grade 3, 12 g). Elution of the column with 2.5, 5, and 10% benzene in hexane gave a total of 204 mg of oil which started to crystallize on standing. Two recrystallizations of the combined fraction from petroleum ether (bp 30–60°) gave pure phenol IV, tentatively formulated as 4-hydroxy-2-methyl-19-norcholesta-1,3,5(10)-triene, as a colorless crystalline solid of mp 124.5–125°, $[\alpha]^{20}D$ +77.1°. An analytical sample was recrystallized again from a small amount of petroleum ether (bp 30–60°) to give colorless rods: mp 125.5–126.5°; ultraviolet spectrum λ_{max} 283 mμ (ε 1800), 277 (1700), 227 (5400); infrared spectrum 3600, 1620, 1580, 1470, 1385, 1305, 1264, 1158, 976 cm^{-1}; in CS_2 845, 825, 752, 715 cm^{-1}; KBr 839 and 825 cm^{-1};[9] nmr spectrum (δ) 2.27 (s, aromatic CH_3), 6.52 and 6.78 (1-CH and 3-CH).[9]

Anal. Calcd for $C_{27}H_{42}O$: C, 84.75; H, 11.07. Found: C, 84.56; H, 10.78.

Fraction 4 (0.65 g) contained *ca.* 25% of the R_f 0.32 compound (4% of total mixture). Column chromatography of the fraction on alumina (grade 3, 20 g) and elution with benzene–hexane (1:3) gave the desired compound (151 mg) which crystallized on standing. Recrystallization from petroleum ether gave 57.6 mg of colorless crystalline solid, mp 119–121°. A second recrystallization from the same solvent gave 2-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene (V) as a crystalline solid: mp 120.5–121°; $[\alpha]^{20}D$ +74.1° (lit.[8] mp 120–120.5°); ultraviolet spectrum λ_{max} 282 mμ (ε 2300), 223 (6200); infrared spectrum 3595, 1610, 1470, 1382, 1315, 1300, 1175, 1140, 1012, 980 cm^{-1}; in CS_2 890, 852, 828, 753, 744, 712 cm^{-1}; nmr spectrum (δ) 2.17 (s, aromatic CH_3), 6.57, 6.72 (1-CH and 3-CH).

Anal. Calcd for $C_{27}H_{42}O$: C, 84.75; H, 11.07. Found: C, 84.95; H, 10.93.

Fraction 5 (1.81 g) was highly enriched in compound R_f 0.28 (40% of total reaction mixture). Crystallization of the product from petroleum ether (bp 30–60°) gave 1.678 g of crystals, mp 125–126.5°. Two additional recrystallizations from petroleum ether gave 3-hydroxy-1-methyl-19-norcholesta-1,3,5(10)-triene (III) as colorless needles: mp 127.5–128°; $\alpha^{20}D$ +141.2° (lit.[7] mp 126.5–128°, $[\alpha]^{20}D$ +135.6°); ultraviolet spectrum λ_{max} 284 mμ (ε 1900), 225 (8300); infrared spectrum 3600, 1614, 1590, 1475, 1385, 1305, 1175, 1142, 970, 860 cm^{-1}; in CS_2 896, 860, 852, 832, 718 cm^{-1}; nmr spectrum (δ) 2.42 (s, aromatic CH_3), 6.80 (m, 2-CH and 4-CH).

Sodium Borohydride Reduction of 1,4-Cholestadien-3-one. A. Kinetic Study.—To each of eight centrifuge tubes was added 8.9 mg of 1,4-cholestadien-3-one. *t*-Butyl alcohol (0.2 ml) was added to dissolve the dienone. From a stock solution of 150 mg of sodium borohydride in 100 ml of *t*-butyl alcohol was pipeted the desired amount of borohydride for each tube (*cf.* Table III). Each tube was then brought to a total volume of 5.0 ml by the addition of *t*-butyl alcohol. The tubes were then stoppered and immediately placed in a constant-temperature bath at 36°. Periodically, 0.2 ml was pipeted from each tube and diluted to 10 ml of absolute ethanol. The uv spectrum was then taken and the optical density of the 244-mμ band recorded. The results are summarized in Table III.

On completion of the rate study, 4 ml of water was added to the solution remaining in each tube. Each mixture was then extracted three times with 2 ml of ether. The ether was evaporated and each residue was spotted on a single tlc plate. The developed plate showed identical patterns for each of the eight tubes, both in R_f values and intensity of spots (five compounds indicated). Glpc of the reaction mixture from tube 8 displayed six peaks at 12.8, 9.0, 4.3, 2.1, 1.3 and 1.1 min. Isolation of five of the main compounds will be described in the preparative-scale experiment.

To a solution of 154 mg (0.4 mmol) of 1,4-cholestadien-3-one in 150 ml of 2-propanol (36°) was added 362 mg (9.6 mmol) of sodium borohydride and the spectral data were recorded (time in hours, OD_{244}): 0, 1.20; 1, 1.15; 3, 0.76; and 20, 0.06. Tlc of the reaction material gave the same pattern of compounds as the chromatogram from the *t*-butyl alcohol reduction.

B. Preparative Run.—To a solution of 2.67 g (7 mmol) of 1,4-cholestadien-3-one in 1.5 l. of *t*-butyl alcohol (36°) in a 2-l. erlenmeyer flask was added 2.12 g (56 mmol) of sodium borohydride. The flask was stirred for a brief period, stoppered and placed in a constant-temperature bath (36°). Aliquots were removed periodically and absorption at λ_{max} 244 mμ was recorded as optical density units (time in hours): 0 (1.60), 16 (0.42), 24 (0.25), 40 (0.10) and 45 (0.07). After the 46-hr reaction period most of the solvent was removed under reduced pressure, 500 ml of water was added and the resulting mixture was extracted three times with 50-ml portions of chloroform. The combined chloroform extracts were washed three times with saline water, dried over sodium sulfate and evaporated under reduced pressure. An additional 5.35 g of dienone was reduced in the same manner. Tlc of the pale yellow crude product showed the presence of five compounds (R_f 0.76, 0.53, 0.42, 0.34, 0.27). The crude mixture (8.0 g) was subjected to column chromatography on alumina (230 g, Woelm grade 1). The fractions obtained are listed in Table IV.

Fraction B-1 contained a nonpolar product (R_f 0.76, 8% of total reaction mixture) besides several more polar compounds. Fraction B-1 was chromatographed on alumina (grade 1, 26 g). Elution with hexane (180 ml) gave 263 mg of colorless oil. On standing at room temperature for a period of 2–3 weeks, the pure oil was apparently partly autoxidized as shown by tlc. The material (263 mg) was then subjected to preparative tlc (two plates, 20 × 20 × 0.5 mm). After development of the plates in hexane–acetone–ether (8:1:1), the top portion of the plates was eluted with acetone to give 109 mg of pure IX as a pale yellow oil, $\alpha^{20}D$ +52.3°. The compound was stored in frozen benzene: ultraviolet spectrum $\lambda_{max}^{n\text{-hexane}}$ 269, 260, 254, 248, 242, 228 mμ; infrared spectrum 1582, 1468, 1382, 1368, 1085, 965, 940, 851 cm^{-1}; nmr spectrum in CCl_4 (δ) 2.15 (s, aromatic CH_3) and 6.75–7.03 (m, aromatic CH) [lit.[12] 2.15 ppm (s) and 6.75–7.05 (m)].

Fraction B-2 (680 mg) which contained two compounds (R_f

Table IV

Fractionation of Reduction Products of Cholestadienone

Fraction	Solvent (vol)	Amount, g
B-1	Hexane to benzene:hexane 1:1 (6 l.)	0.87
B-2	Benzene (1.5 l.)	0.68
B-3	Ether–benzene 1:9 (1.5 l.)	1.20
B-4	Ether–benzene 1:3 (1.5 l.)	0.57
B-5	Ether–benzene 1:1 (1.5 l.)	1.09
B-6	Ether (1.5 l.)	1.77
B-7	1% methanol in ether (1.5 l.)	0.81
B-8	2.5–10% methanol in ether (4.5 l.)	0.44
B-9	25% MeOH in ether (1.5 l.)	0.17
B-10	Chloroform–ethyl acetate–methanol 1:1:1 (1.5 l.)	0.08

0.53 and 0.42) was chromatographed on alumina (grade 1, 20 g) and eluted with benzene–hexane (1:3) to give 101 mg of colorless crystals, R_f 0.53 (10% of total crude yield), pure by tlc. Recrystallization from petroleum ether gave 61.7 mg of colorless crystals, mp 98–106. Further recrystallization from petroleum ether gave 5β-cholest-1-en-3-one (VI) as colorless rectangles, mp 107–108°, $[\alpha]^{20}D$ +135.9° (lit.[10] mp 104°).

An additional recrystallization from the same solvent did not alter the melting point: ultraviolet spectrum λ_{max} 233 mμ (ε 8700); infrared spectrum 1675, 1468, 1378, 1270, 1112, 975, 840 cm^{-1}; nmr spectrum (δ) 6.98, 6.80, 6.03, 5.87 (AB quartet).

Anal. Calcd for $C_{27}H_{44}O$: C, 84.31, H, 11.53. Found: C, 84.02, H, 11.33.

Further elution of the fraction B-2 column with benzene gave 120 mg of colorless crystals (R_f 0.42, 18% total reaction mixture). Two recrystallizations of the material from petroleum ether gave 72.3 mg of 4-cholesten-3-one (VII) as clusters of needles: mp 82–82.5°, $\alpha^{20}D$ +93.2°; ultraviolet spectrum 239 mμ (ε 15,600). A mixture melting point of the sample with authentic 4-cholesten-3-one was undepressed at 81.5–82.5°. The infrared spectrum of VII was identical with that of the authentic sample.

Fraction B-6 (1.77 g) after chromatography on alumina (grade 1, 45 g) gave compound R_f 0.27 (40% of total reaction mixture). Elution of the column with ether–benzene (1:3) gave 585 mg of purified material (tlc). Crystallization of the product from acetone–methanol gave 460 mg of 4-cholesten-3β-ol (VIII) as colorless needles: mp 128–130°; $[\alpha]^{20}D$ +49.3 (lit.[11] mp 130–132°, $\alpha^{20}D$ +46°). The mixture melting point with authentic sample was undepressed and the infrared spectra were identical.

Fraction B-3 (1.20 g) contained the desired R_f 0.34 compound (12% of total reaction mixture) together with 4-cholesten-3-one (VII). Column chromatography of this fraction on alumina (grade 1, 36 g) and elution with 4% ether in benzene gave 101 mg of highly purified compound (tlc). Recrystallization of this material from petroleum ether gave 71 mg of colorless crystals: mp 114–115°; $[\alpha]^{20}D$ +31.2°; λ_{max} 244 mμ (ε 16,800), identical with starting material I by mixture melting point (no depression) and comparison of infrared spectra.

Irradiation of 1,4-Cholestadien-3-one in the Presence of Sodium Borohydride. A. Kinetic Studies.—A solution of 267 mg (0.7 mmol) of 1,4-cholestadien-3-one in 50 ml of *t*-butyl alcohol (36°) was added to a solution of 212 mg of sodium borohydride in 100 ml of *t*-butyl alcohol (36°). The mixture was immediately placed in the irradiation chamber. Aliquots were removed periodically and absorption at λ_{max} 244 mμ was recorded. Time is given in minutes (optical density units): 0 (1.70); 5 (1.15), 15 (0.62), 30 (0.17) and 60 (0.10). After 75 min, the solution was added to 300 ml of water. The mixture was extracted three times with 50 ml of chloroform. The combined extracts were washed several times with saline water and then dried over sodium sulfate. Evaporation of the solvent gave 290 mg of crude product as a viscous pale yellow oil. Tlc of the reaction mixture showed the presence of five compounds. Glpc showed the presence of six main peak areas at retention times of 1.1 min (minor), 2.1 (major), 3.0 min (major), 4.1 (minor), 4.9 min (major) and 5.8 min (major). For isolation of five of these compounds; *cf.* preparative-scale experiment.

A solution of 154 mg (0.4 mmol) of 1,4-cholestadien-3-one in 100 ml of 2-propanol (36°) was added to a solution of 362 mg (9.6 mmol) of sodium borohydride in 50 ml of 2-propanol (36°). The mixture was irradiated and the ultraviolet absorption was recorded as previously described (time in minutes, OD): 0, 1.20; 5, 0.98, 15, 0.48; 30, 0.14; 60, 0.07. Examination of the reaction mixture by tlc showed identical compounds (R_f and intensity) in comparison with the tlc of the *t*-butyl alcohol reaction product. 2-Propanol irradiation products were not isolated.

B. Preparative Run.—A solution of 5.34 g (14 mmol) of 1,4-cholestadien-3-one in 1 l. of *t*-butyl alcohol (36°) was added to a solution of 4.24 g (115 mmol) of sodium borohydride in 2 l. of *t*-butyl alcohol (36°). The resulting solution was immediately added to the irradiation chamber. Aliquots of the solution were removed periodically during the irradiation period in order to record ultraviolet absorption data as previously described (time in minutes, OD_{244}): 5, 1.50°, 20, 1.20; 40, 0.80; 80, 0.30; 120, 0.14; 150, 0.11. After the reaction was quenched by the addition of 100 ml of water, most of the solvent evaporated under reduced pressure. To the concentrated mixture was added 1 l. of water, followed by extraction with three 75-ml portions of chloroform. The combined chloroform extracts were washed with water, saline water, and then dried over sodium sulfate. Evaporation of the solvent gave 5.45 g of tan solid. The above reaction was repeated to give a total amount of 10.89 g of irradiated material. Tlc of the product showed the presence of one nonpolar compound and four closely related polar compounds (R_f 0.76, 0.53, 0.44, 0.40, 0.36) and several substances near the origin in trace amounts. The crude mixture (10.89 g) was chromatographed on alumina (grade 1, 475 g). The various fractions obtained are summarized in Table V.

Table V

Fractionation of Products Resulting from the Irradiation of Cholestadienone in the Presence of Borohydride

Fraction	Solvent (vol)	Amount, g
BH-1	Hexane (2 l.)	0.06
BH-2	Benzene–hexane (1:9) to ether (16 l. total)	1.67
BH-3	1% methanol in ether (2 l.)	4.02
BH-4	2.5% methanol in ether (2 l.)	2.11
BH-5	5% methanol in ether to chloroform–ethyl acetate–methanol (8 l. total)	2.234

Fraction BH-2 contained the nonpolar R_f 0.76 compound (5% of total crude mixture) together with more polar substances. Approximately 250 mg of this fraction was applied to two preparative tlc plates (20 × 20 × 0.5 mm). The chromatograms were developed with hexane–acetone–ether (8:1:1) and the upper portion of the plates eluted to give a total of 72 mg of pale yellow oil, $[\alpha]^{20}D$ +41.4°. The fraction was dissolved in benzene and the solution frozen to prevent autoxidation of the compound. The R_f value (tlc) of the compound was identical with that of hydrocarbon IX: ultraviolet spectrum λ_{max}^{hexane} 269 mμ (ε 310), 260 (570), 254 (700), 248 (650), 242 (760), and 230 (1110). The nmr spectrum was identical with that of IX. The mass spectrum of this compound showed M^+ 366, *m/e* 364, *m/e* 351 ($M^+ - CH_3$), and *m/e* 211 (ring C fragmentation).

Fraction BH-3 (4.02 g) was chromatographed on 120 g of alumina (grade 3) and eluted with benzene–hexane (1:9) to give 820 mg of thick oil which consisted largely of the R_f 0.53 compound (15% of total). Attempts to crystallize this compound from two solvent systems failed. The material was then rechromatographed on alumina (25 g, grade 3). Elution with benzene–hexane (1:9) gave 68 mg of colorless crystals which were recrystallized from petroleum ether to give 11.4 mg of colorless crystals, mp 142–144°. A second recrystallization from the same solvent gave pure 1-hydroxy-4-methyl-19-norcholesta-1,3,5(10)-triene (II) as needles, mp 145–146°. The compound was identical with phenol II obtained from the irradiation experiment as shown by mmp 145–147° and identical infrared spectra.

Further elution of the BH-3 column with benzene–hexane (1:3) gave 1.0 g of material containing the R_f 0.40 compound (8% of total reaction mixture) contaminated by two other compounds (tlc). This material was rechromatographed on alumina (30 g, grade 3). Elution with 1, 2.5, 5, and 10% benzene in hexane gave a combined fraction of 531 mg as a

thick yellow oil. Rechromatography of this fraction with grade 2 alumina and elution with benzene–hexane (1:1) and benzene gave 243 mg of the desired compound as a colorless solid contaminated by a compound with lower R_f as shown by tlc. Final purification of the compound was achieved by two preparative tlc procedures. In the final procedure, 96 mg of the mixture was placed on two preparative tlc plates (20 × 20 × 1 mm silica gel G plates containing fluorescein dye). The plates were developed continuously for 6 hr (hexane–acetone–ether, 9:0.5:0.5). Elution of the top uv zone from each plate gave a combined yield of 44 mg of colorless solid. Recrystallization of the solid from methanol gave 21.6 mg of colorless crystalline clusters, mp 108–112°. A second recrystallization from methanol gave 17.3 mg (X): mp 112–113.5°; $[\alpha]^{20}D$ −45.3°; infrared spectrum 3618, 1470, 1385, 1114, 1068, 1032, 1020, 918, 832 cm^{-1}; nmr spectrum (δ) 5.25 (m, olefin), 1.26 (s, C-19 methyl), 0.67 (s, C-18 methyl).

Further elution of the BH-3 column with benzene–hexane (1:1) gave 997 mg of impure compound R_f 0.44 (13% of total). The material was then rechromatographed two times on alumina. The final procedure on elution with benzene and ether–benzene (1:9) (grade 2 alumina) gave a combined yield of 325 mg of product highly enriched in the desired compound, but not crystalline. The material was therefore applied to three preparative tlc plates (1-mm plates containing fluoroscein dye). After continuous development for 3 hr and uv lamp inspection of the plates, the lower portion of the top uv zone was eluted with acetone to give a combined yield of 147 mg. The product was recrystallized from petroleum ether to give two crops of colorless crystals, 20.7 mg, mp 122–123°, and 61.0 mg, mp 125–125.5°. The combined crops were recrystallized from petroleum ether to give 68.4 mg of 4-hydroxy-2-methyl-19-norcholesta-1,3,5(10)-triene (IV), as colorless crystals, mp 124.5–125.5°. The compound was identical with IV obtained from the irradiation experiment as shown by mmp 125–126.5° and infrared and ultraviolet spectra.

Fraction BH-4 (2.11 g) was subjected to column chromatography on alumina (grade 1, 63 g). Elution with 0.1, 0.25 and 0.5% methanol in ether gave 1.03 g of combined material containing the desired compound, R_f 0.36 (45% of total reaction mixture), accompanied by minor impurities. The material was then rechromatographed two more times on alumina, the final procedure (17 g, grade 3) after elution with benzene–hexane (1:1) gave 220 mg of pure product. Recrystallization from petroleum ether gave colorless fine needles, mp 126.5–127.5°. A second recrystallization from the same solvent gave pure 3-hydroxy-1-methyl-19-norcholesta-1,3,5(10)-triene (III), mp 128–128.5°. The compound was identical with III obtained from the irradiation experiment with regard to infrared spectrum and mmp 126.5–127.5°.

Registry No.—I, 566-91-6; II, 19202-72-3; III, 17605-79-7; IV, 19202-74-5; V, 19202-75-6; VI, 19202-76-7; IX, 2603-79-4; sodium borohydride, 1303-74-8.

Steroids. LXXX.[1] The Effect of C-12 Substitution on the Reactivity of Δ^{16}-20-Keto Steroids toward 1,4-Nucleophilic Addition[2,3]

G. SHUFORD ABERNETHY, JR., AND MONROE E. WALL

Chemistry and Life Sciences Laboratory, Research Triangle Institute, Research Triangle Park, North Carolina 27709

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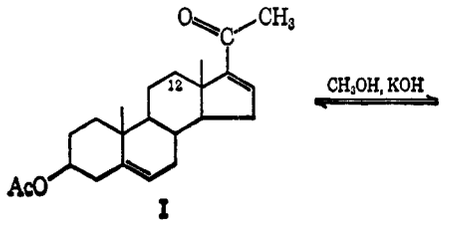
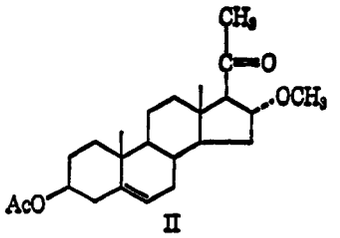
The presence of a ketone function at C-12 has a marked rate accelerating effect on 1,4 additions to Δ^{16}-20-keto steroids. The rates of the base-catalyzed 1,4 addition of methanol to a wide variety of 12-substituted Δ^{16}-20-keto steroids have been measured. On the basis of nmr and ultraviolet spectral data it is concluded that the rapid rate of reaction exhibited by the 12-keto steroids is anomalous. A mechanism is proposed to explain the unexpected rate of 1,4 addition displayed by Δ^{16}-12,20-diketo steroids. This effect is shown to be applicable to a number of nucleophiles, some of which undergo further reaction to produce polycyclic derivatives.

In 1951, Fukushima and Gallagher[4] characterized the product obtained from the action of methanolic potassium hydroxide on Δ^{16}-pregnenolone acetate (I) as the methanol 1,4-addition product II.



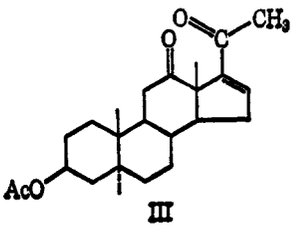
I

CH_3OH, KOH



II

Mueller, *et al.*,[5] have shown that the presence of a ketone at C-12 greatly increases the rate of this reaction. Adams, *et al.*,[6] have also observed this effect and attributed the increased reactivity to the polar effect of the 12-ketone on the adjacent conjugated system. In an earlier publication[7] we reported the facile base-catalyzed 1,4 addition of acetone to the 12-keto compound III.[8] The 12-deoxy analog I[9] failed to



III

react with acetone under the same conditions. It occurred to us that the increased reactivity of III could

(1) Previous paper in this series (Steroids. LXXIX): C. E. Cook, R. C. Corley, and M. E. Wall, *J. Org. Chem.* **33**, 2789 (1968).

(2) (a) The research in this paper was supported under Contract SA-43-ph 4351 of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health. (b) Presented at the 19th Southeastern Regional Meeting of the American Chemical Society, Atlanta, Ga., Nov, 1967.

(3) Taken from the M.S. Thesis of G. S. Abernethy, Jr., North Carolina State University, 1967.

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