BILE ACIDS. LXVIII. ALLYLIC AND BENZYLIC PHOTO-CHEMICAL OXIDATION OF STEROIDS[1]

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Received 5-23-83

ABSTRACT

To provide 7-oxocholesterol derivatives in yields superior to those obtained by chemical oxidation, the preparation of steroidal allylic or benzylic ketones was studied. Air-induced oxidation was investigated with a highly selective low energy UV lamp in the presence of mercuric bromide. With this procedure cholesteryl acetate, 5-cholestene-3β,27-diol diacetate, 24(R)-24-methyl-5-cholesten-3β-yl acetate, 24(R)-24-ethyl-5-cholesten-3β-yl acetate and 24(R)-24-ethyl-(22E)-cholesta-5,22-dien-3β-yl acetate were oxidized to the allylic keto-derivative in good yields; estradiol-17β diacetate was similarly converted to the 6-oxo-product in improved yield. This method can be very useful in the synthesis of 7-oxocholesteryl acetate and its analogs and 6-oxo-estratrienes.

INTRODUCTION

Recognition that the first committed step in the biosynthesis of bile acids from cholesterol is the formation of 7α-hydroxycholesterol by a hepatic microsomal enzyme system (3,4) has stimulated an interest in the development of better synthetic methods for the production in quantity of 7-oxygenated sterols (5). The formation and identification of such materials from the free sterol via autooxidation (6) and/or irradiation (7-9) has been a topic of interest since at least 1908 (10), although from a preparative point of view the yield of products is generally poor. Chemical methods have included allylic oxidation of choles-

teryl acetate, e.g., chromic anhydride (10,11) or t-butyl chromate (12,13), neither of which affords a high yield of the 7-oxo derivative (10). A simplified procedure of Friedman et al. (14) for the production of 7-oxocholesteryl acetate in good yield by photooxidation of cholesteryl acetate in the presence of a source of bromine atoms at 254 nm has now been examined in greater detail. This paper reports improved results in the formation of 7-oxo derivatives of several sterols and an estrogen after modification of the procedure to include irradiation with a low pressure lamp in the presence of a flow of air or oxygen.

RESULTS AND DISCUSSION

Preliminary studies with a Hanovia immersion lamp (UV-medium pressure mercury vapor) established certain parameters for the conversion of cholesteryl acetate (Ia) to the 7-oxo derivative (IIa). The polarity of the solvent (entries 1-4 in Table I) was not important in the formation of desired product in high yield, but mercuric bromide was essential (entry 2) for the reaction to occur. If the concentration of reactant and of $HgBr_2$ was increased twelve times (entry 4), the yield was markedly reduced; mercurous bromide was precipitated continuously during the reaction to form a cloudy suspension. Continued irradiation after filtration of the suspension afforded continued precipitation of mercurous bromide. The energy of the Hanovia lamp could not be controlled to maintain the reaction mixture at room temperature, so the system was discarded in favor of a lamp of lower energy.

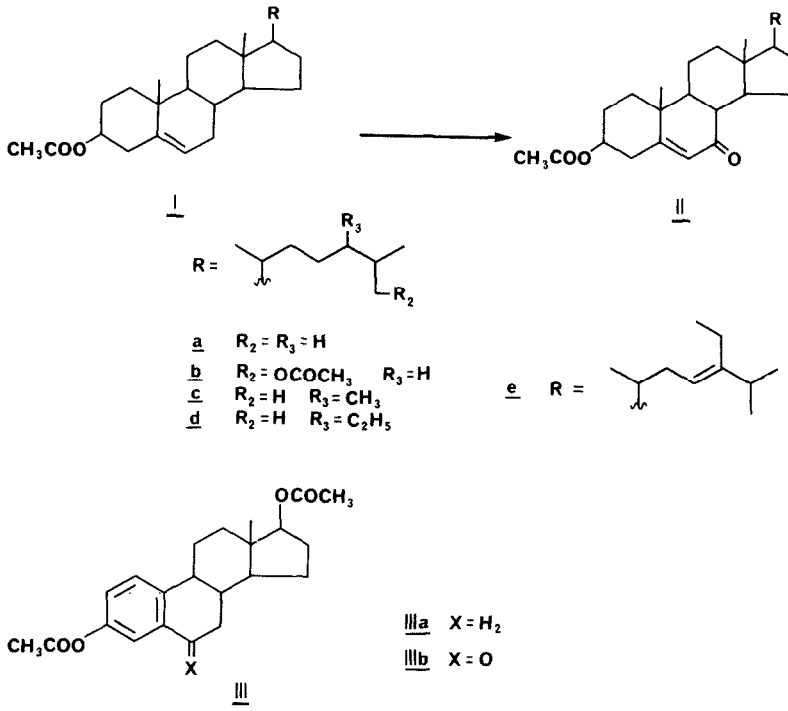
A Pen-Ray immersion lamp (low pressure mercury vapor, Ace Glass Co.) which provides most of the radiation at 254 nm without raising the temperature of the reaction medium was utilized satisfactorily in all of the following studies. 7-Oxocholesteryl acetate was obtained in 80% or

Table I

Conditions and Yields of Allylic and Benzylic Photo-oxidation

Entry	Compound	Conc.[1]/Solvent[2]	Lamp[3]/System[4]	Gas Phase	Time (h)	Product	Yield (%)
1	Cholesteryl acetate	1/Cy	H/A	air	2.5	7-Oxocholesteryl acetate	95
2	" "	1/Cy	H/A[5]	air	4.5	" "	0
3	" "	1/t-B	H/A	air	3.5	" "	94
4	" "	12/t-B	H/A	air	5.3	" "	12
5	" "	1/t-B	P/B	air	5	" "	80
6	" "	6/t-B	P/B	air	22	" "	99
7	" "	5.4/t-B	P/B	---	24	" "	22
8	5-Cholestene-3β,27-diol diacetate	6.8/t-B	P/B	air	25	7-Oxo-5-cholestene-3β,27-diol diacetate	89
9	Cholesteryl acetate	5.4/t-B	P/C	air	1	7-Oxocholesteryl acetate	82
10	" "	5.4/t-B	P/C	O_2	1	" "	78
11	" "	5.4/t-B	P/C	air	24	" "	0
12	24(R)-24-Methyl-5-cholesten-3β-yl acetate	3/t-B	P/C	air	2	7-Oxo-24(R)-24-methyl-5-cholesten-3β-yl acetate	90
13	24(R)-24-Ethyl-5-cholesten-3β-yl acetate	3.3/t-B	P/C	air	1.5	7-Oxo-24(R)-24-ethyl-5-cholesten-3β-yl acetate	85
14	24(R)-24-Ethyl-(22E)-cholesta-5,22-dien-3β-yl acetate	5.7/t-B	P/C	air	3	7-Oxo-24(R)-24-ethyl-(22E)-cholesta-5,22-dien-3β-yl acetate	62
15	" "	5.7/EtAc	P/C	air	2.3	" "	tr[6]
16	Estradiol-17β diacetate	2.6/t-B	P/C	air	2	6-Oxo-estradiol-17β diacetate	36
17	" "	2.5/EtAc	P/C	air	1.67	" "	41

(1) in g/l; (2) t-B = t-butanol; Cy = cyclohexane; EtAc = ethyl acetate; (3) H = Hanovia Immersion UV-medium pressure mercury vapor lamp (450 W).; P = Pen-Ray, low pressure mercury vapor lamp (2.5 W); (4) A = a reaction vessel cooled externally by ice, lamp in a quartz jacket cooled with a water/methanol mixture (7:1) to about 40°C and with a Vycor glass filter (2 mm layer); B = a 500 ml, three necked, round bottomed flask, at room temperature; C = an irradiation tube at room temperature; (5) without $HgBr_2$; (6) only traces of starting material and 7-oxo derivative could be found in gas chromatography. Yields were determined by glc.



quantitative yield (entries 5 and 6) after 5 or 22 hrs irradiation, respectively, even though the latter reaction medium was at 6 fold concentration. Without a flow of air (entry 7) only 22% of product was detected after 24 hrs of irradiation.

To ascertain the effects on the reaction of substituents in the sterol side chain, the diacetate of 27-hydroxycholesterol diacetate (Ib) (11) was utilized as a substrate (entry 8). The 7-oxo derivative (IIb) was obtained in 89% yield (average of 5 experiments). With this reactant, again the importance of bubbling air through the medium during irradiation was demonstrated; without a flow of air only a very low yield of product (IIb) was obtained. After the unreacted substrate from

this experiment was recovered, mixed with fresh t-butanol and $HgBr_2$ and irradiated with air bubbling through the medium, the reaction proceeded with high yield of product. With a solvent mixture of isooctane-t-butanol (77.5:12.5), a continuous precipitation of mercurous bromide occurred with a small yield of product. Reirradiation of recovered reactant under the proper conditions above (entry 8) provided the product in high yield.

To investigate other aspects of this procedure, reactions were carried out with the Pen-Ray immersion lamp placed in a tubular vessel fitted at the bottom with a fritted disc for dispersion of the gas bubbled through the system (see System C in EXPERIMENTAL for details). The reaction time in this tube was reduced drastically. Thus, irradiation of cholesteryl acetate (entry 9) for one hr with 1.5 mole-equivalent of mercuric bromide in t-butanol with an air flow of about 10 ml/min provided 82% of the product (Ib).

Repetition of this experiment for 45 min with ethyl acetate as solvent provided similar results with only a slightly lower yield. With oxygen at a flow rate of 12.5 ml/min (entry 10), no significant change in yield was seen. The course of this reaction for 100 min is given in Fig. 1; after about 10 min of induction or saturation, the product was produced at a rate of 2-2.5% per min until a plateau was reached in about one hr. Another product was formed at the same time at a slower rate. After 24 hr, only a mixture of more polar compounds was detected with no evidence of starting material or desired product (entry 11).

Homologs of cholesteryl acetate, particularly campesteryl acetate (15) (Ic) and β-sitosteryl acetate (Id), were found to provide the putative products in good yield, subsequently identified as the 7-oxo

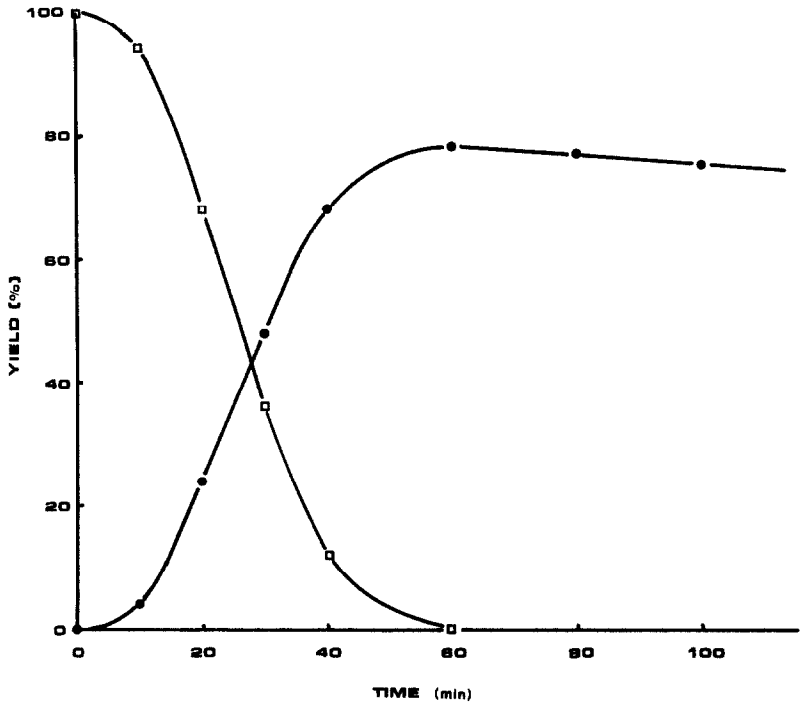


Figure 1. Time course of allylic photo-oxidation reaction of cholesteryl acetate (□) with $HgBr_2$ and oxygen in t-butyl alcohol; product, 7-oxo-cholesteryl acetate (●).

derivatives (entries 12 and 13). With stigmasteryl acetate (Ie) as reactant in t-butanol, the 7-oxo product (IIe) was obtained after irradiation for three hr (entry 14). With ethyl acetate as solvent, the reaction proceeded with loss of reactant and formation of only traces of the desired product (entry 15; after 140 min only traces of either were detected by glc). Since ethyl acetate was an effective solvent in allylic oxidation of cholesteryl acetate, the double bond in the side chain may be responsible for the poor yield of desired product.

Estradiol-17β diacetate (IIIa) was selected as a reactant to study benzylic oxidation in this system. After 2 hrs of irradiation in t-butanol (entry 16) 36% of 6-oxo-estradiol-17β diacetate (IIIb) was obtained. After 1.67 hrs in ethyl acetate the yield was 41% (entry 17). Figure 2 depicts the progress of the former reaction (entry 16) with time. The concentration of the reactant decreased linearily with time (slope, -0.54±0.02). In this first order reaction, two uncharacterized products were formed in equal amounts (10%) after 2 hrs.

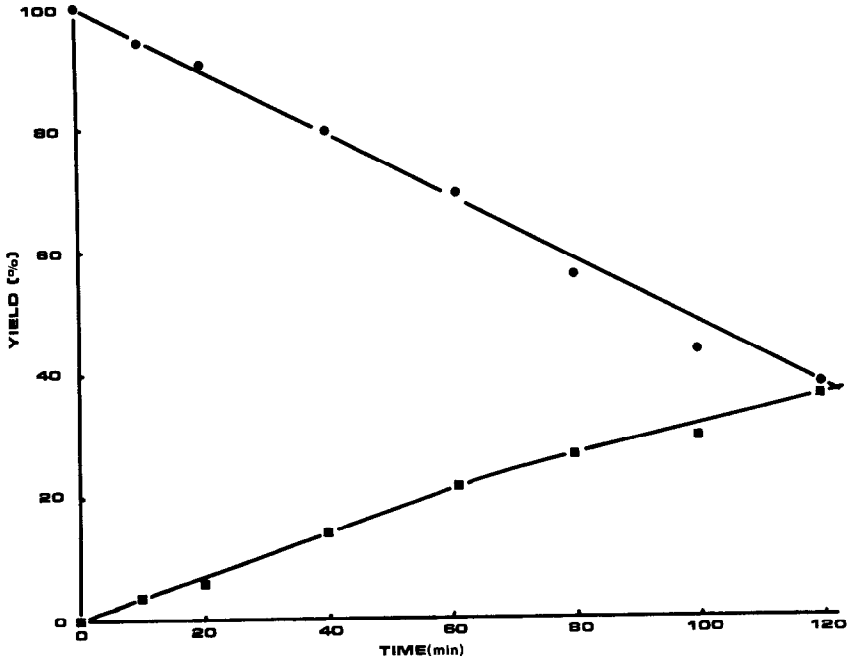


Figure 2. Time course of the benzylic photo-oxidation reaction of estradiol-17β diacetate (●) with $HgBr_2$ and air in t-butanol; product, 6-oxo-estradiol-17β diacetate (■).

Thus, with a lamp of lower energy for irradiation and the modifications introduced here, this method has been useful in providing products of allylic and benzylic oxidation in good yield. The details of these modified procedures for a model compound are given below.

EXPERIMENTAL

Materials and Methods: All solvents were analytical grade or were redistilled. The photo-oxidation reactions were carried out in three different systems: (A) A Canrad-Hanovia photochemical immersion lamp (medium pressure, quartz, mercury vapor, 450 W; Ace Glass Co., Vineland, NJ 08360, Model 7825-34) was fitted into a double-walled quartz immersion well (Ace Glass Co., Model 7854B-26) containing a mixture of water-methanol (7:1) chilled externally by a Lauda circulating pump (Model K2-R) to -10°C; a Vycor glass filter (2 mm layer, Ace Glass Co, Model 7835-40 which provides 68% of the radiation at 254 nm) was attached, and the entire system was placed in the reaction vessel (Ace Glass Co., Model 7847) which contained the solution of reactant and a glass frit attached to the bottom through which a dry stream of air was passed during the reaction. To control the temperature of the solution containing the reactant, the entire system was placed in an ice bath; however, the temperature of the mixture in the immersion well was generally about 40° despite these efforts.

(B) A 500 ml three-necked round bottom flask was fitted with a magnetic stirrer, a Pen-Ray photochemical immersion lamp (U.V. low pressure, 2.5 W, which delivers 80-90% of the radiation at 254 nm without the use of filters or external cooling; Ace Glass Co., Model 12130-06); a glass fritted tube (Corning Glass, Corning, NY, No. 39533-12C) for gas dispersion, and a loosely fitting thermometer.

(C) A Pyrex irradiation tube (23 mm i.d. x 20 cm) fitted with a ₹29/42 joint at the top, a fritted glass tube (9 mm i.d., medium porosity) at the bottom, and an open side arm attached at about a 30° angle 20 mm below the joint for passage of released gas to a flowmeter. The gas used in reaction was passed through the glass frit and thence through the reaction medium during irradiation. The Pen-Ray immersion lamp described above was inserted into the irradiation tube and held in place with a ₹24/40 - 29/42 bushing adapter.

The yield of products was measured by glc on 3% OV-1 as follows: column, 260°; detector and flash heater, 275° (16). Characterization of all reactants and products of these reactions has been reported (11,12, 15,19,20,21). Curves similar to Fig. 1 were obtained for each reactant. Identification of products was confirmed by physical characterization. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Mass spectrometry and gas chromatography-mass spectrometry were carried out with an LKB Model 9000 (16,17). Analytical tlc plates were performed on aluminum plates coated with 0.2 mm thickness silica gel (MCB Manufacturing Chemists, Inc., Cincinnati, OH). Preparative HPLC (18) was carried out with a Water Associates Prep LC/System 500 on

Prep-Pak 500/Silica cartridges. Infrared spectra were recorded on a Model 21 Perkin-Elmer double beam spectrophotometer as KBr pellets.

Photochemical oxidations. For large amounts of substrate, in gram quantities, system B was used. In a typical experiment (entry 6 in the Table) cholesteryl acetate (2.1 g, 4.9 mmol) and mercuric bromide (2.64 g, 7.33 mmol) dissolved in 350 ml of t-butanol were irradiated for 22 hrs, with a stream of air (dried by a $CaCl_2$ tube) at a flow rate of 10-15 ml/min. The cloudy solution was filtered from the mercurous bromide, concentrated in an evaporator and the remaining oil was extracted three times with water-methylene chloride. The combined organic solutions were washed three times with saturated aqueous NaCl solution, dried over $MgSO_4$, filtered and evaporated to yield 2.20 g of crude material (99% 7-oxocholesteryl acetate by glc; RRT (1) = 2.19). Crystallization from ethyl acetate afforded colorless crystals, mp = 159-160°C (reported (19) 156-158°C).

For experiments on a small scale the irradiation tube described in system C was used with the following procedure (entry 9 in the Table). A solution of cholesteryl acetate (214 mg, 0.5 mmol) and mercuric bromide (267 mg, 0.74 mmol) in 40 ml of t-butanol was irradiated while a stream of dried air (8-12 ml/min) was bubbled through it. The progress of the reaction was checked by glc and tlc from samples taken at different times. After 60 min only traces of starting material remained and the yield of 7-oxocholesteryl acetate was 82%. Its physical properties (tlc, glc, MS) were identical to those of an authentic sample.

The products, except entries 16 and 17, exhibited λ_{max} 229-230 nm (ether), were all located on the chromatographic plate after the development by their UV absorption, and were found to have mobilities uniformly less than the reactant when developed in ethyl acetate:hexane (1:9 or 2:8). The mass spectrum of 7-oxo-5-cholestene-3β,27-diol diacetate (entry 8) was comparable to that of an authentic sample (11, 12). Fragment ions reported by Aringer and Nordström (20) for 7-oxo-[24R]-24-methyl-5-cholesten-3β-yl acetate and 7-oxo-[24R]-24-ethyl-5-cholesten-3β-yl acetate were found in spectra of the products reported as entries 12 and 13, respectively. Particularly diagnostic in spectra of those products containing the 5-en-7-one conjugated system are the fragment ions m/z 174, 242 and 269 retaining this system (11,12). The structure of 7-oxo-[24R]-24-ethyl-(22E)-cholesta-5,22-dien-3β-yl acetate (19) was also supported by glc and tlc; the reaction product showed the same RRT and the same R_f as a commercial sample. 6-Oxo-estradiol-17β diacetate (21) was characterized by λ_{max} 245 nm (ether), RRT (0.88) and its mass spectrum. The fragment ions m/z 370 (M^+, 2%), 328 (M-42, 100%), 310 (M-60, 3.3%), 286 (M-84, 8.4%) and 266 (M-104, 20.2%) are 14 mass units larger than the corresponding fragment ions in the spectrum of estradiol-17β diacetate; the ion corresponding to M-42 is base peak in the spectrum of each component.

ACKNOWLEDGEMENTS

This investigation was supported by U.S.P.H.S. Grant H1-07878. The technical assistance of G. Douglas Frisch, David Brown and John Crimi in

mass spectrometry and/or the preparation of several intermediates for this study is gratefully acknowledged. The Canrad-Hanovia lamp was kindly made available by Dr. Vincent T. Spaziano of the Department of Chemistry, St. Louis University.

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1. The following abbreviations have been used: UV, ultra-violet; tlc, thin layer chromatography; glc, gas liquid chromatography; HPLC, high performance liquid chromatography; RRT, relative retention time (relative to cholesteryl acetate).
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