[CONTRIBUTION FROM THE METCALF CHEMICAL LABORATORIES OF BROWN UNIVERSITY]

Chemical and Kinetic Studies on the Reaction of β-Cholestanyl p-Toluenesulfonate with Alcohols and with Sodium Alkoxides in Alcohols

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Chemical and kinetic studies of the reaction of β-cholestanyl p-toluenesulfonate with methanol and with sodium methoxide in methanol show that the reactions are first order in the steroid and unaffected by the addition of methoxide ion. An ionization mechanism involving backside attack by solvent on an unstable 2°-carbonium ion is proposed. A similar study on the reaction with t-butyl alcohol and with sodium t-butoxide in t-butyl alcohol indicates that a bimolecular mechanism is also operating in this case.

In a previous study[1] the reaction of β-cholestanyl p-toluenesulfonate with methanol was found to yield 17% of a one-to-one mixture of Δ^2- and Δ^3-cholestene and 73% of α-cholestanyl methyl ether. No evidence could be obtained for the formation of any of the β-methyl ether, and the reaction thus appears to involve replacement of the tosylate group by backside attack of methanol or methoxide ion.

In order to gain further information on the reaction path, chemical and kinetic studies have been made of the effect of bases on the decomposition of the β-cholestanyl tosylate.

Chemical Studies

When the β-cholestanyl tosylate was heated in methanol for 72 hours with varying amounts of sodium methoxide, no change in the product composition was observed within the limits of experimental error. These results are shown in Table I.

Replacement of the methanol by t-butyl alcohol yielded 37% of the Δ^2-, Δ^3-cholestene mixture, 11% of α-cholestanyl t-butyl ether and 23% of α-cholestanol. The reaction was slower than with methanol, and 12% unreacted tosylate was recovered. The α-cholestanol was apparently formed by cleavage of the α-cholestanyl t-butyl ether by the p-toluenesulfonic acid produced in the reaction.

When sodium t-butoxide was added to the reaction, a slight increase in the ratio of olefin to ether was observed. No α-cholestanol was formed presumably because the p-toluenesulfonic acid produced in the reaction was neutralized by the sodium t-butoxide. The results are given in Table I.

TABLE I

REACTION OF β-CHOLESTANYL TOSYLATE WITH VARIOUS REAGENTS FOR 72 HOURS AT REFLUX TEMPERATURE

Reagent	Yield Δ^2-, Δ^3-cholestene, %	Yield of α-cholestanyl ether, %	Ratio of olefin to ether (average)
Methanol	17	73	0.23
Methanol and 4 equiv. NaOMe	20–21	67–74	.29
Methanol and 8 equiv. NaOMe	19–23	74–77	.28
t-Butyl alcohol	34–37	22–36[a]	1.10
t-Butyl alcohol and 4 equiv. $NaOC(CH_3)_3$	41–43	27–33	1.41
t-Butyl alcohol and 8 equiv. $NaOC(CH_3)_3$	39–47	26–30	1.54

[a] Yield is adjusted to allow for α-cholestanol.

(1) H. R. Nace, THIS JOURNAL, **74**, 5937 (1952).

An experimental difficulty was encountered in the reactions in t-butyl alcohol, in that chromatography on alumina did not effect clean-cut separation of the olefins and ether. However, oxidation with potassium permanganate decomposed the olefins in the mixture and made possible isolation of the α-cholestanyl t-butyl ether. From the rotations of the pure ether and the olefins it was then possible to calculate the amounts of these compounds in the mixture.

The structure of the ether was established by cleavage with aqueous alcoholic hydrochloric acid which yielded 67% of α-cholestanol. No β-cholestanol could be detected in the product. If present, it should have been found easily since it is readily separated from the α-epimer by chromatography on alumina.

Kinetic Studies

The rate and order of the methanol–tosylate reaction were determined by titration of the p-toluenesulfonic acid produced. A first-order plot resulted in a straight line for about 70% of the reaction, thus showing the reaction to be first order in β-cholestanyl tosylate.

The effect of the addition of 1 and of 2.5 equivalents of sodium methoxide was determined in a similar manner by addition of standard acid to an aliquot withdrawn from the hot solution and back titration of the unconsumed acid with base. Again, a first-order plot (first order in β-cholestanyl tosylate) gave a straight line in both cases, and the rate constants were in good agreement with the ones obtained in the absence of sodium methoxide. The results are shown in Table II.

TABLE II

RATE CONSTANTS FOR THE REACTION OF β-CHOLESTANYL TOSYLATE WITH ALKOXIDES AT THE REFLUX TEMPERATURE

10^2 moles/l. tosylate	10^2 moles/l. NaOMe	$10^3 k$, sec.$^{-1}$	10^2 moles/l. tosylate	10^2 mole/l. $NaOC(CH_3)_3$	$10^3 k$, sec.$^{-1}$
0.280		1.1	0.222	0.222	0.5
.185		1.1	.220	.220	0.2
.242	0.242	1.0	.224	.554	1.2
.255	.255	1.3	.205	.523	1.0
.236	.590	1.3			
.236	.590	0.9			

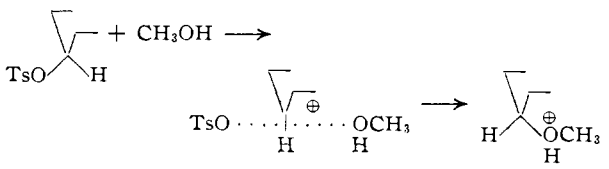
Kinetic studies of the reaction of the β-cholestanyl tosylate with t-butyl alcohol were not possible because of the cleavage reaction with the p-toluenesulfonic acid. However, studies were made

of the effect of the addition of 1 and of 2.5 equivalents of sodium *t*-butoxide on this reaction.

Due to the experimental difficulties encountered in the titrations of aliquots, it was not possible to determine accurately the rate or the order of the reaction. However, the rough measurements indicated that increasing the amount of base increased the rate of the reaction.

Discussion of Results

The chemical and kinetic results show clearly that in the reactions of the tosylate in methanol, the substitution is accompanied by complete inversion, and is unaffected by the addition of methoxide ion. The reaction appears, therefore, to be an example of a solvolysis with complete inversion. An example of such a reaction was reported recently by Streitwieser,[2] who found that the solvolysis of butyl 1-*d*-brosylate was accompanied by complete inversion. His picture of the reaction appears to be applicable to the case reported here. The rate-determining step is the solvolysis of the tosylate to give a "covalently solvated" secondary carbonium ion, in which the solvating species are the tosylate ion and methanol. Immediate reaction of this intermediate (because of its instability) with the solvating methanol would lead to complete inversion, and such a picture would accommodate the fact that



methoxide ion has no effect on the rate of the reaction. The accompanying first-order elimination reaction would take place in a similar fashion, except that the methanol would remove an α-hydrogen by solvation instead of solvating the carbon carrying the tosylate group.

Other examples of solvolysis of a tosylate being unaffected by the addition of the conjugate base have been reported, notably by Robertson[3] who found that the solvolysis of isopropyl benzenesulfonate in 50% alcohol–water was unaffected by the addition of hydroxide ion.

The picture in the case of the reactions with *t*-butyl alcohol and sodium *t*-butoxide is less clear. The reaction is slower than in the case of methanol, complete inversion still takes place, but the addition of the alkoxide ion affects the reaction by increasing the rate and changing the product composition in favor of a higher proportion of olefin to ether.

Neither a first-order nor a second-order plot of the kinetic data gave a straight line and the evidence suggests that both the ionization mechanism given above and a bimolecular mechanism of elimination and substitution are operating. Because of the experimental difficulties mentioned above it has not been possible, as yet, to obtain clear-cut kinetic evidence that both mechanisms are operating.

(2) A. Streitwieser, THIS JOURNAL, **77**, 1117 (1955).

(3) R. E. Robertson, *Can. J. Chem.*, **31**, 589 (1953).

Experimental[4]

β-Cholestanyl tosylate was prepared as described previously,[1] m.p. 136–137° dec., $[\alpha]D$ +6°.

Reaction of β-Cholestanyl Tosylate with Sodium Methoxide in Methanol.—To a solution of 52 mg. (2.2 milligram atoms) of sodium in 35 ml. of methanol (Baker and Adamson, ACS Reagent Grade) was added 300 mg. (0.55 millimole) of β-cholestanyl tosylate. The solution was heated under reflux for 72 hours and then the solvent was removed under reduced pressure and the residue was taken up in 50 ml. of ether and 50 ml. of water. The ether layer was successively washed with water, dilute sodium carbonate solution, water and saturated sodium chloride solution and then filtered through anhydrous sodium sulfate. The residue (210 mg.) was chromatographed as described previously[1] to separate the Δ^2- and Δ^3-cholestene mixture from the α-cholestanyl methyl ether. The yields are given in Table I.

The same procedure was used for the reaction with eight equivalents of sodium methoxide and the yields are given in Table I.

Reaction of β-Cholestanyl Tosylate with *t*-Butyl Alcohol.—A solution of 300 mg. (0.55 millimole) of β-cholestanyl tosylate in 40 ml. of *t*-butyl alcohol (b.p. 82–83°, distilled from calcium hydride) was heated under reflux for 72 hours. The reaction mixture was worked up as above and the residue (236 mg.) was chromatographed on 10 g. of alumina. Petroleum ether eluted a mixture of Δ^2- and Δ^3-cholestenes (1:1 mixture) and α-cholestanyl *t*-butyl ether. The yields given in Table I were calculated from the rotations of the mixture and of the pure compounds (see below).

Ether eluted a mixture (131 mg.) of α-cholestanol and unreacted β-cholestanyl tosylate. The ether residue was crystallized from acetone to give 52 mg. (23%) of α-cholestanol, m.p. 185–185.5°, $[\alpha]D$ +32°. The mother liquor was evaporated to dryness and the residue (68 mg., $[\alpha]D$ +7°) (22.7%) was crystallized from acetone–water to give 35 mg. (12%) of β-cholestanyl tosylate, m.p. 130–132°, $[\alpha]D$ +6°.

Reaction of β-Cholestanyl Tosylate with Sodium *t*-Butoxide in *t*-Butyl Alcohol.—A solution of 300 mg. (0.55 millimole) of β-cholestanyl tosylate in 35 ml. of *t*-butyl alcohol containing 52 mg. (2.2 milligram atoms) of sodium was heated under reflux for 72 hours and then the reaction was worked up as above. The residue was chromatographed on 10 g. of alumina.

Petroleum ether eluted a mixture of Δ^2- and Δ^3-cholestenes (1:1 mixture) and α-cholestanyl *t*-butyl ether. The yields are given in Table I.

Ether eluted unreacted β-cholestanyl tosylate (12%).

The reaction with eight equivalents of sodium *t*-butoxide was carried out in the same manner and the yields are given in Table I.

α-Cholestanyl *t*-Butyl Ether.—To a solution of 75 mg. ($[\alpha]D$ +37°) of a mixture of Δ^2- and Δ^3-cholestenes and α-cholestanyl *t*-butyl ether (obtained as described above) in 30 ml. of acetone were added 1 ml. of 1% aqueous potassium permanganate solution and 1 ml. of 6 *N* sulfuric acid. The solution was allowed to stand for one hour at room temperature and then 100 ml. of ether was added. The ether solution was washed successively with water, dilute sulfuric acid, water and saturated sodium chloride solution, filtered through anhydrous sodium sulfate, and the ether evaporated. The residue was chromatographed on 10 g. of alumina and petroleum ether eluted 36 mg. of α-cholestanyl *t*-butyl ether, m.p. 92.5–94.5°, $[\alpha]D$ +20°. Several recrystallizations from acetone gave an analytical sample, constant m.p. 95.5–96.5°, constant $[\alpha]D$ +19°.

Anal. Calcd. for $C_{31}H_{56}O$: C, 83.78; H, 12.61. Found: C, 83.97; H, 12.42.

Cleavage of α-Cholestanyl *t*-Butyl Ether to α-Cholestanol.—A solution of 50 mg. of α-cholestanyl *t*-butyl ether in 30 ml. of ethanol containing 20 drops of 6 *N* hydrochloric acid was heated under reflux for 3 hours. Then the solution was concentrated to 10 ml., water was added, and the solid was collected by filtration and dried. The crude material (m.p. 155–170°, 46 mg.) was chromatographed on 10 g. of

(4) All melting points are corrected; rotations at room temperature, approximately 1% chloroform solutions. Merck and Co., Inc., alumina (suitable for chromatographic absorption) was used for chromatography. Analysis by Dr. S. M. Nagy and associates, Microchemical Laboratories, Massachusetts Institute of Technology.

alumina. Benzene eluted 29.4 mg. of α-cholestanol, which gave, after one recrystallization from aqueous acetone, 29 mg. (67%), m.p. 184–185°, $[\alpha]_D$ +31°.

Elution with ether yielded no β-cholestanol, which, if present, should be obtained at this point.[5]

Kinetic Studies

β-Cholestanyl Tosylate in Methanol.—The weighed sample of β-cholestanyl tosylate in a Pyrex capsule was dropped into the boiling methanol, and the resulting solution heated under reflux. Periodically 5-ml. aliquots were withdrawn and titrated with 0.02 *N* sodium ethoxide in ethanol, using phenolphthalein indicator. During the first 12 hours of reaction samples were taken every hour and then every 5 or 6 hours. The results are shown in Table II.

(5) F. Galinovsky and O. Vogl, *Monatsh.*, **79**, 325 (1948).

For the reactions with sodium methoxide in methanol, solutions of sodium methoxide in methanol were standardized just before use. The procedure was similar to the one above except that the aliquots were pipeted into known amounts of standard solutions of potassium acid phthalate in ethanol and the resulting solutions back titrated with standard sodium ethoxide in ethanol. The results are shown in Table II.

Reaction of β-Cholestanyl Tosylate with Sodium *t*-Butoxide in *t*-Butyl Alcohol.—The procedure was similar to the one above for sodium methoxide in methanol. However, the end-points in the titrations were not sharp and were masked by the opalescence of the solutions which developed during the titrations. It was also difficult to get reproducible results in the several runs studied, and for these reasons the values given in Table II must be considered as only approximate.

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[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Steroidal Cyclic Ketals. XX.[1] 16-Hydroxylated Steroids. III.[2] The Preparation of 16α-Hydroxyhydrocortisone and Related Compounds

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Hydroxylation of 16-dehydroprogesterone (I) with osmium tetroxide yielded 16α,17α-dihydroxy-progesterone (IIa). In a similar manner by hydroxylation of the corresponding Δ^{16}-compounds (free steroid or 21-acetate derivatives), there were obtained 16α-hydroxy-Reichstein's substance S (VII), 16α-hydroxycortisone (XIa) and 16α-hydroxyhydrocortisone (XIIIa). Various acetate derivatives of these 16-hydroxylated compounds were also characterized. An alternative pathway to compounds VII, XIa and XIIIa proceeded *via* $\Delta^{5,16}$-bis-ethylene ketal intermediates.

Various investigations[3] on the corticosteroids present in the adrenal venous blood of a variety of species have indicated, by paper chromatographic techniques, the presence of substances more polar than hydrocortisone. The determination of the exact nature of these substances presents a difficult problem. Furthermore, in this connection, certain workers[4] have elicited interest in "corticoids" possessing additional hydroxyl groups at the 6- and/or 16-positions. Concurrent to these developments, we initiated a project on the chemical preparation of a number of such hydroxylated "corticoids." Our efforts were first directed to the synthesis of compounds in which a 16α-hydroxyl group was present and, in particular, to the synthesis of 16α-hydroxyhydrocortisone (XIIIa).[5] A discussion of the preparation of the latter and other related compounds forms the basis of this paper.

In a recent paper[6] from this Laboratory, a novel pathway was described for the preparation of a number of $\Delta^{4,16}$-3,20-diketosteroids and their corresponding 3,20-bis-ethylene ketal derivatives. These two types of compounds served in this investigation as key intermediates for the desired 16α-hydroxylated steroids.

16-Dehydroprogesterone ($\Delta^{4,16}$-pregnadiene-3,20-dione) (I) (Flowsheet A) was selected as a model substance for this work. Thus, compound I dissolved in benzene (containing pyridine), on treatment with osmium tetroxide was converted into 16α,17α-dihydroxy-progesterone (α^4-pregnene-16α,-17α-diol-3,20-dione) (IIa) (47% yield).[7,8] Acetylation under mild conditions afforded the 16α-acetate IIb. Considerations bearing on the structure of IIa and the other 16α-hydroxylated steroids will be presented collectively later in the paper.

The diol IIa on ketalization with ethylene glycol in the conventional manner gave in poor yield Δ^5-

(1) Paper XIX, R. H. Lenhard and S. Bernstein, THIS JOURNAL, **78**, 989 (1956).

(2) Paper II, S. Bernstein, M. Heller and S. M. Stolar, *ibid.*, **77**, 5327 (1955).

(3) See O. Hechter and G. Pincus, *Physiol. Revs.*, **34**, 459 (1954), and references cited therein.

(4) R. I. Dorfman and F. Ungar, "Metabolism of Steroid Hormones," Burgess Publishing Co., Minneapolis, Minn., 1953, p. 13, and references cited therein; see also, M. Hayano and R. I. Dorfman, *Arch. Biochem.*, **50**, 218 (1954).

(5) In a previous paper [S. Bernstein, M. Heller and S. M. Stolar, THIS JOURNAL, **76**, 5674 (1954)], there was reported the *chemical* preparation of Δ^5-pregnene-3β,16α-diol-20-one and 16α-hydroxyprogesterone.

(6) W. S. Allen and S. Bernstein, *ibid.*, **77**, 1028 (1955).

(7) Examples of hydroxylation of a Δ^{16}-double bond with osmium tetroxide have been described by A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **24**, 1127 (1941), and by V. Prelog, L. Ruzicka and P. Wieland, *ibid.*, **28**, 250 (1945). Also the preparation of IIa from 16-dehydroprogesterone (I) by selective hydroxylation with osmium tetroxide has been reported by H. H. Inhoffen, F. Blomeyer and K. Brückner, *Chem. Ber.*, **87**, 593 (1954). We wish to state here that our preparation of IIa, and, in fact, of all the 16α-hydroxylated steroids described herein was completed prior to the appearance of the publication of the German workers.

(8) No difficulty was encountered in effecting the selective hydroxylation of the Δ^{16}-double bond in a $\Delta^{4,16}$-3,20-diketosteroid (four examples) (30–72% yield). A 9–48% excess of osmium tetroxide was employed, and the reactions were allowed to take place for the arbitrary time of 1–5 days. The hydroxylation generally appeared to proceed rapidly. However, opportunity was not available for a definitive time study of the reaction.

Moreover, the Δ^{16}-double bond in a $\Delta^{5,16}$-3,20-bis-ethylene ketal (three examples) may also be selectively hydroxylated (45–94% yield, 3–20% excess of osmium tetroxide, 3–7 days). Here, also, a time study of the reaction was not carried out.