66-5; VIb, 10498-67-6; VIc, 10498-68-7; VIIa, 10498-69-8; VIIb, 10498-70-1; VIII, 10498-71-2.

Acknowledgment.—We wish to acknowledge the interest and support of Dr. T. F. Gallagher throughout this investigation. We are grateful to Mrs. Beatrice S. Gallagher for the determination and interpretation of the infrared spectra, to Mrs. Julia Liang for the nmr spectra, and to Millie Smulowitz for invaluable technical assistance.

Stereochemistry and Kinetic Isotope Effects in the Formolytic Rearrangement, Substitution, and Elimination Reactions of Androsterone *p*-Toluenesulfonate[1]

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The formolysis of 3α-*p*-toluenesulfonoxy-5α-androstan-17-one gave mainly 5α-androst-2-en-17-one and lesser amounts of the Δ^3 isomer and of the formates of 3β-, 2β-, 3α-, and (in traces) 2α-hydroxy-5α-androstan-17-one. The four formates are listed in the declining order of their yields after correction is made for the slow addition of formic acid to the olefin which gave mainly the 3α and 2β, some of the 2α, and traces of the 3β isomers. The rearrangement of the 3α-tosylate to the 2β-formate proceeds in part (about 20%) *via* the 2α-tosylate which was isolated after an incomplete reaction. The formolysis of the latter was 0.35 times as fast as that of androsterone tosylate and gave, beside olefins, the 2β-formate. The 2β-tritiated androsterone tosylate lost less tritium on formolysis (6%) than the 2α-tritiated analog (23%). This indicates that the elimination reaction of the (axial) 3α-tosylate was predominantly *cis*. Its rearrangement to the 2β-formate, however, proceeds mainly by the migration of the 2β-hydrogen. This unusual steric result, retention of configuration at C-2, is attributed to the shielding effect of the departing tosylate ion, since such a mechanism would also accommodate the lesser pathway to the 2β-formate *via* the 2α-tosylate. Kinetic isotope effects were calculated for the formation of the main reaction products. When the starting tosylate was axially tritiated at C-2, the 3β-formate showed an isotope effect, k_H/k_T, that was significantly lower than that for the disappearance of the tosylate or those for the formation of the 2β- and 3α-formates and of the mixture of olefins. The implications of these results are compared with previously suggested schemes for the solvolysis of *cis*-4-*t*-butylcyclohexyl tosylate. In the additions of formic and of trifluoroacetic acid to the Δ^2-olefin, the entry of the nucleophilic agent was predominantly from the α side and thereby differed from the course of other ionic addition reactions of 5α-steroidal Δ^2-olefins.

After the initial work of Stoll[2] in 1932, solvolytic reactions of 3-tosyloxy steroids were investigated many times. In the absence of the Δ^5 double bond and of vicinal substituents, the course of the substitution reaction was found to be uniform and resulted in a product of inverted configuration at C-3. This was accompanied by varying amounts of olefin. This course was observed, for example, with equatorial tosylates in acetic acid containing acetate ion,[3] and with both axial and equatorial tosylates in pyridine,[4] piperidine,[4] and alcohols.[5,6] The methanolysis of the equatorial 5α-cholestan-3β-ol tosylate is of particular interest because Pappas, *et al.*,[7] found no acceleration of the rate upon the addition of sodium methoxide and concluded that a reaction *via* a carbonium ion had yielded only the methyl ether of inverted configuration. The conversion of androsterone sulfate to 2α- and 2β-hydroxy steroids on acid hydrolysis[8] suggested the existence of reaction paths from a C-3 cation which had not been observed when such a carbonium ion was generated from the tosylate. In searching for rearrangement products of the tosylate it seemed best to use a solvent of greater ionizing power and lower nucleophilic characteristics than methanol. The present report describes the solvolysis of androsterone *p*-toluenesulfonate[9] in formic acid.

To minimize possible effects of toluenesulfonic acid or its anion,[10] we conducted the formolysis at great dilution (0.0005 *M*). It proceeded at a good rate at room temperature and gave after alkaline hydrolysis of the formates, the products shown in Table I, runs 2 and 3.[11] As in the hydrolysis of androsterone sulfate (Table I, run 1) all four isomers hydroxylated at C-2 and C-3 (**4**, **5**, **6**, and **8**) were obtained but in significantly different yields, because the 2β-hydroxy compound was now the main alcoholic product and the yields of the 2α and 3α isomers were relatively diminished. Only part of the formate fraction was obtained from the tosylate by a direct route, because the olefin undergoes a slow addition reaction. A solution of the androstenone recovered from the formolysis (which was about a 9:1 mixture of the Δ^2 and Δ^3 isomers) and of an equimolar amount of *p*-toluenesulfonic acid in formic acid gave, after a reaction time of 163 hr and after alkaline hydrolysis of the formates, the four alcohols in yields shown in run 4 of Table I. The reaction appears to be irreversible because the 2β-formate which presumably is the least stable of the four isomers failed to give

(1) Supported by U. S. Public Health Service Grants CA 01679 and AM 09105 and a Research Career Program Award K6-AM-14367. Some of the results have been given in a preliminary report: J. Ramseyer and H. Hirschmann, *Federation Proc.*, **24**, 534 (1965).

(2) W. Stoll, *Z. Physiol. Chem.*, **207**, 147 (1932).

(3) P. A. Plattner and A. Fürst, *Helv. Chim. Acta*, **26**, 2266 (1943).

(4) L. C. King and M. J. Bigelow, *J. Am. Chem. Soc.*, **74**, 3338 (1952).

(5) H. R. Nace, *ibid.*, **74**, 5937 (1952); J. R. Lewis and C. W. Shoppee, *J. Chem. Soc.*, 1375 (1955).

(6) R. Gardi, R. Vitali, and A. Ercoli, *Gazz. Chim. Ital.*, **92**, 632 (1962).

(7) N. Pappas, J. A. Meschino, A. A. Fournier, and H. R. Nace, *J. Am. Chem. Soc.*, **78**, 1907 (1956).

(8) J. Ramseyer, J. S. Williams, and H. Hirschmann, *Steroids*, **9**, 347 (1967).

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(10) A. Streitwieser, Jr., T. D. Walsh, and J. R. Wolfe, Jr., *J. Am. Chem. Soc.*, **87**, 3682 (1965); A. Streitwieser, Jr., and T. D. Walsh, *ibid.*, **87**, 3686 (1965).

(11) In run 3, which was conducted under standard conditions, a very small amount of benzene and of acetone was used to give an instantaneous solution of the tosylate in formic acid which dissolves crystals of the tosylate only on prolonged shaking. The very similar yields of run 2, which was made without these cosolvents, serve to show that the results were not decisively influenced by the use of acetone as might be argued from the observations of H. Weiner and R. A. Sneen, *ibid.*, **87**, 287 (1965).

TABLE I

PERCENTAGE YIELDS OF REACTION PRODUCTS[a]

No.	Reaction[b]	Products				
		Olefin	3α-OH-A (5)	3β-OH-A (4)	2α-OH-A (8)	2β-OH-A (6)
1	3α-SuO-A + HCl-H_2O	72.5	10.6	3.3	1.2	0.9
2	3α-TsO-A (1) + HCOOH	87.2	2.6	3.6	0.3	4.6
3	3α-TsO-A (17b, 18b) + HCOOH	87.6	2.4	4.1	0.3	4.4
		±1.1	0.0	0.1	0.0	0.0
3a	Same, cor[c] for addn	90.7	1.2	4.0	0.1	3.5
4	Olefin + HCOOH (163 hr)[d]	73.4	9.6	0.4[e]	2.0	7.9
		±0.4	0.3		0.2	0.0
5	Olefin + CF_3COOH (48 hr)	+[f]	45.5	1.9	4.7	16.1

[a] Abbreviations: A = 17-oxo-5α-androstanyl; Su = $HOSO_2$-; Ts = *p*-toluenesulfonyl. Products of reactions 2–5 were isolated after alkaline hydrolysis. [b] Reaction 1 is described elsewhere.[8] Reactions 3 and 4 were done under standard conditions, reaction 2 with commercial formic acid, without cosolvents as described in the Experimental Section. Reaction 4 contained 1 molar equiv of *p*-toluenesulfonic acid. The olefin used for reaction 4 was isolated from reaction 3, the one used for 5 was obtained from 2. Reactions 3 and 4 were done with labeled steroids (derived from **17a** and **18a**) and yields are the means of two runs (± deviations) calculated from the ^{14}C counts as percentages of those put on the column. The other runs were done with unlabeled steroids and yields were determined by weight. For further details, see the Experimental Section. [c] Equation 14 in the Appendix. [d] The 2β-hydroxy isomer was preceded on the column by a peak of radioactivity. This unidentified material may be 4β-hydroxy-5α-androstan-17-one. [e] Single measurement made by dilution of the ^{14}C counts in sample by 6.5 mg of unlabeled **4** and determination of the specific activity. [f] Unidentified mixture described in the Experimental Section.

olefin or androsterone formate under the conditions of the addition reaction.[12] The yields of alcohols which were used to correct for the addition reaction are the means of four experiments, the two long-term runs (interpolated values for total yield, 2.7 and 2.9%) and two that were run for the duration of the solvolysis reaction (20 hr) and which showed a much greater variation in rate (total yields 2.4 and 4.0%). The corrected values (entry 3a) demonstrate that the 2β, 3α, and 3β isomers are formed by processes besides addition to the double bond but do not justify this conclusion for the 2α compound. Although the isotope data given below indicate that some of the 2α-formoxyandrostanone is actually formed by substitution, it is clear that the rearrangement which produces 2-oxygenated steroids by formolysis of the tosylate is highly stereoselective.

While a rearrangement of a 3α-tosylate to a 2β-hydroxy steroid or its ester has not been reported before, our findings are analogous to those of Winstein and Holness[13] who studied the formolysis of the *cis* and *trans* isomers of 4-*t*-butylcyclohexyl tosylate and obtained from the axial tosylate 87% olefin and (after hydrogenolysis of the esters with lithium aluminum hydride) approximately equal amounts of both isomers of 4-*t*-butylcyclohexanol as well as of the *trans*-3-*t*-butylcyclohexanol. In contrast, the equatorial *trans*-4-*t*-butylcyclohexyl tosylate gave essentially only olefin and the 4-*t*-butylcyclohexanol with the inverted configuration. Similar results were obtained on acetolysis of the tosylates. To explain the different behavior of the axial and of the equatorial tosylate, the formation of the product with retained configuration from the axial tosylate, and the steric course of its rearrangement, Winstein and Holness postulated that the axial tosylate gave rise to two hydrogen-bridged intermediates which when attacked by solvent underwent the usual diaxial opening of the three-membered ring.

(12) This was deduced from the virtually unchanged infrared spectrum of a sample of 2β-formoxy-5α-androstan-17-one that was kept as an 0.0005 *M* solution in the presence of 1 molar equiv of *p*-toluenesulfonic acid in formic acid (standard conditions, 159 hr). The amount of 5α-androst-2-en-17-one that might have been present was estimated to be less than 0.3%. Moreover, the proportions of the four isomers derived from long- and short-term additions agreed closely.

(13) S. Winstein and N. J. Holness, *J. Am. Chem. Soc.*, **77**, 5562 (1955).

The postulated reaction sequence as it would apply to androsterone tosylate (**1**) is shown in Scheme I and would account for both of the unusual products, androsterone (**5b**) and 2β-hydroxy-5α-androstan-17-one (**6b**). (For complete structures of **1** and **6**, see Scheme II). A mechanism which involves only the first of the two hydrogen-bridged species (**2**) was proposed later by Cram and Tadanier[14] on the basis of their studies of certain aliphatic tosylates. In their scheme, the bridged species would undergo *cis* opening to yield structures corresponding to the 2β and 3β isomers, whereas some of the latter and all of the 3α compound formed from nonbridged ions. This mechanism for the rearrangement was adopted by Bergstrom, *et al.*,[15] for the formation of a 9α-fluoro from a 11β-hydroxy steroid with hydrogen fluoride in pyridine. More recently, Cram's proposal was criticized by Brown, *et al.*,[16] because it involves the opening of a bridge with retention of configuration at the site of attack.

The observation[17] that a uranediol 17a-tosylate is an intermediate in the formolytic rearrangement of a 5α-pregnanediol 20β-tosylate suggested that the high stereoselectivity of the rearrangement of androsterone tosylate might be attributed to the intermediate formation of the tosylate (**7**) of 2α-hydroxy-5α-androstan-17-one. Examination of the products of an incomplete formolysis was hampered by the instability of the tosylates on adsorption chromatography and by the inefficiency of partition chromatography in their separation. The latter procedure, however, served to remove other products and fractional recrystallization gave both unchanged androsterone tosylate and another tosylate which had a spectrum indistinguishable from that of a sample of 2α-hydroxy-5α-androstan-17-one tosylate (Figure 1). The reference compound was prepared from the parent alcohol (**8**) with tosyl chloride and pyridine. The 2α-tosylate conformed to the pattern observed by Winstein and Holness[13] for equatorial cyclohexyl tosylates and gave 77% olefin and 20% of

(14) D. J. Cram and J. Tadanier, *ibid.*, **81**, 2737 (1959).

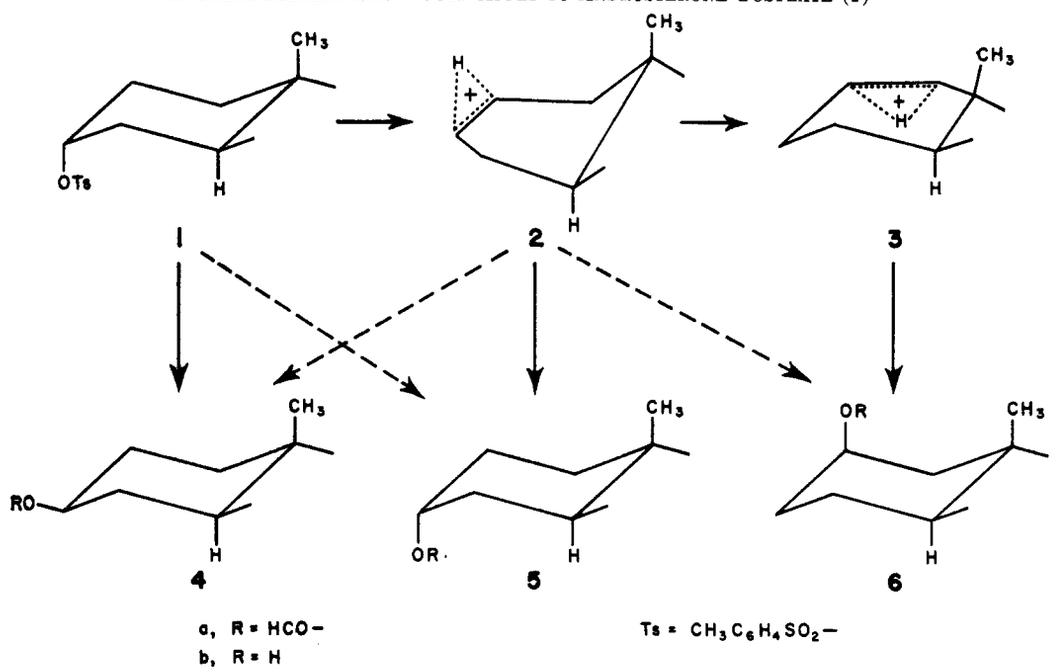
(15) C. G. Bergstrom, R. T. Nicholson, and R. M. Dodson, *J. Org. Chem.*, **28**, 2633 (1963).

(16) H. C. Brown, K. J. Morgan, and F. J. Chloupek, *J. Am. Chem. Soc.*, **87**, 2137 (1965).

(17) H. Hirschmann, F. B. Hirschmann, and A. P. Zala, *J. Org. Chem.*, **31**, 375 (1966).

SCHEME I

PREVIOUSLY PROPOSED PATHWAYS OF SOLVOLYSIS FOR AXIAL CYCLOHEXYL TOSYLATES AS THESE MECHANISMS WOULD APPLY TO ANDROSTERONE TOSYLATE (1)[a]



[a] Solid arrows, proposal of Winstein and Holness;[13] broken arrows, modification proposed by Cram and Tadanier.[14]

SCHEME II

FORMATION AND REACTION OF 2α-TOSYLOXY-5α-ANDROSTAN-17-ONE

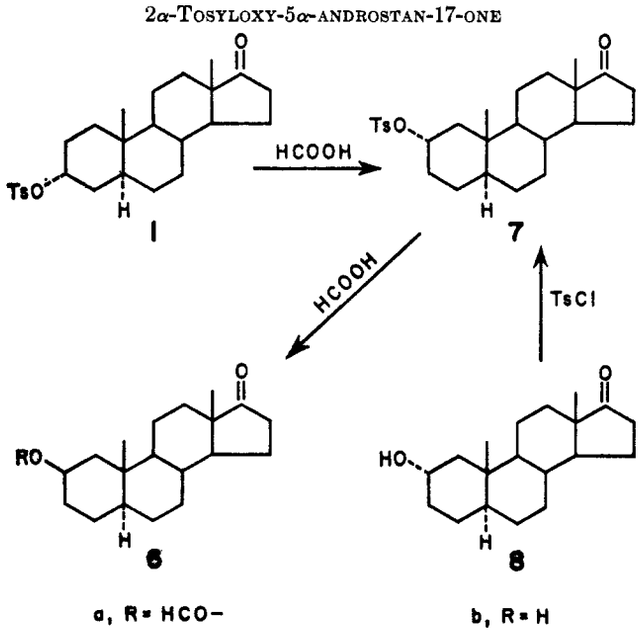


TABLE II

KINETIC MEASUREMENTS

Time × 10^{-2}, sec	Rates[a] × 10^5, sec^{-1} k_s	k (found)	k (calcd)	2α-Ts-A/3α-Ts-A,[b] (7)/(1) Found	Calcd
36	5.14	13.6	13.9		
72	5.41	13.3	13.8		
144	4.97	13.3	13.5		
216	4.83	14.2	13.0	0.382	0.395
288		14.4	12.5	0.863	0.839
360		11.4	11.8		

[a] At 25.0°: k, rate of dissociation of tosylate ion from the mixture of tosylates 1 and 7 that results from the formolysis of 1 [$k = (1/t) \ln (a/a - x)$, where a represents the initial concentration of 1 and $a - x$ is the sum of the concentrations of 1 and 7 remaining after the time t]; k_s, rate of dissociation of the tosylate ion from the tosylate 7. A mean value of 5.09×10^{-5} sec^{-1} was determined for k_s in a separate experiment with 7 as the starting compound of the formolysis. Equations given by Winstein and Schreiber (numbered 10–12)[19] allow one to calculate the rate k as well as the ratio 7/1 for various time intervals from the constants k_s, k_r (the rate of isomerization of 1 → 7), and k_p (the rate of dissociation of the tosylate ion from 1, which equals k at time 0). The rates k_r 0.55×10^{-5} and k_p 14.0×10^{-5} sec^{-1} appeared to give the best fit with the observed tosylate ratios and k values and were used to obtain the calculated figures given in this table. [b] For abbreviations, see Table I.

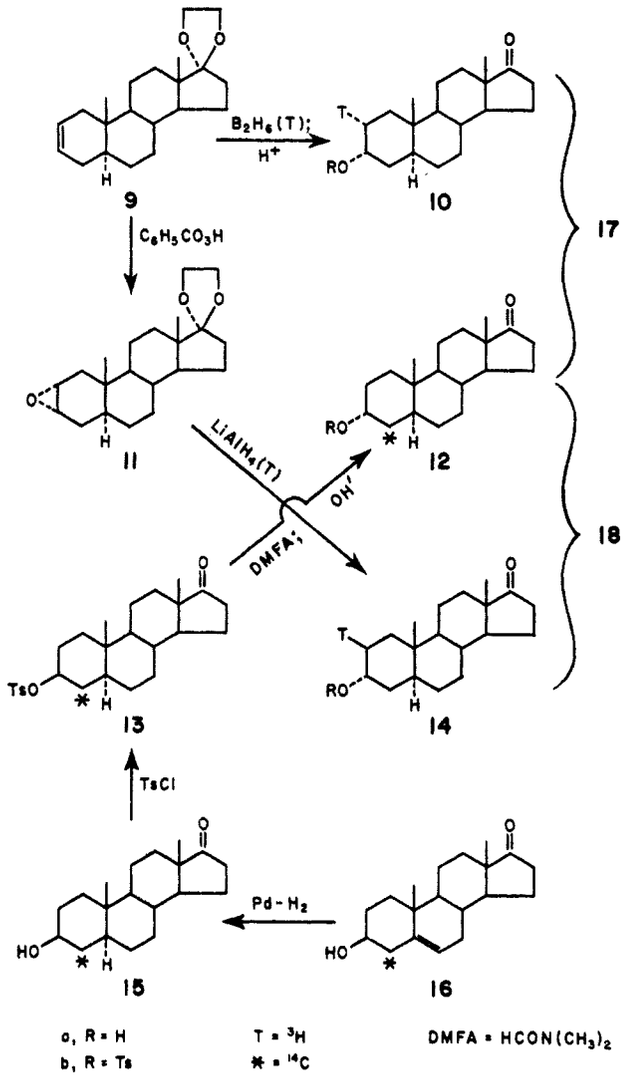
the 2β-formate (6a) as the main substitution product. The yields of isomeric side products were too small to be measured accurately and apparently were no larger than could be expected from addition reactions. The formation and the behavior of the 2α-tosylate, therefor, provides an explanation for the stereoselective formation of the 2β-formate from the 3α-tosylate.

In order to gauge the importance of this pathway, we measured the rates of the formolysis of androsterone tosylate (1) and of 2α-hydroxy-5α-androstan-17-one tosylate (7) by ultraviolet spectroscopy.[17] The rate of formolysis of androsterone tosylate was about 2.8 times as fast as that of the equatorial 2α-tosyloxy compound (Table II). A smaller rate ratio (2.0) has been reported[18] for the acetolysis at 75° of the corresponding 5α-cholestane derivatives. This effect of solvent and temperature is very similar to one reported by Winstein and Holness[13] for the 4-*t*-butylcyclohexyl tosylates. The measurements of the rate of tosylate disappearance lacked the accuracy needed to demonstrate the conversion to a less reactive tosylate. The rate of this process could be estimated, however, by isolating the tosylate fraction after two intervals of an incomplete reaction and by determining the ratio of the two isomeric tosylates by infrared spectroscopy (Table II).

(18) S. Nishida, *J. Am. Chem. Soc.*, **82**, 4290 (1960).

SCHEME III

PREPARATION OF LABELED ANDROSTERONES



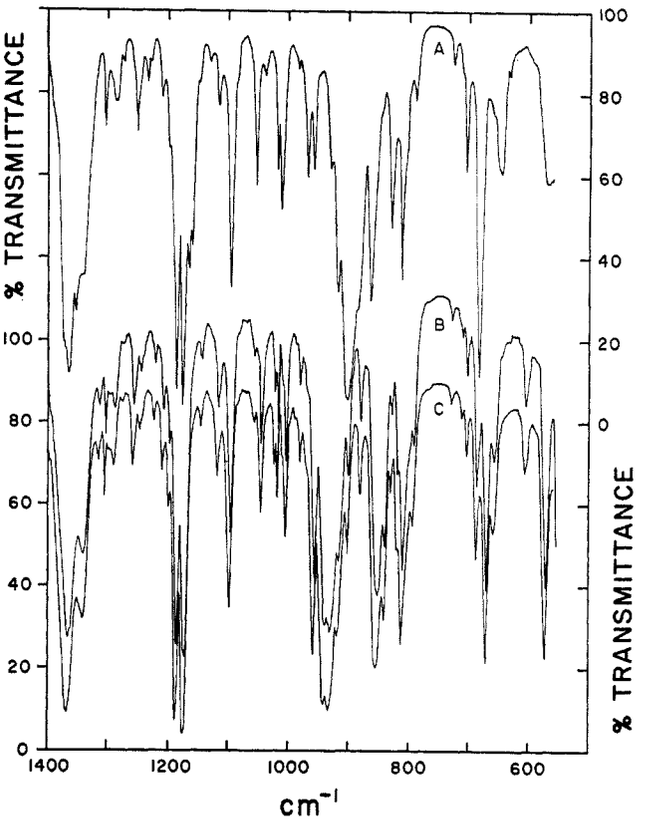


Figure 1.—Infrared spectra (A) of 3α-tosyloxy-5α-androstan-17-one (1), (B) and (C) of 2α-tosyloxy-5α-androstan-17-one (7). B was obtained by formolysis of **1**, C was obtained by tosylation of **8**. Concentrations were 1 mg/0.1 ml; cell length was about 1 mm. Curve A was plotted with respect to the ordinate at the right, C to the left. Curve B was displaced by +20% transmittance from C.

The results were analyzed by the equations that have been given by Winstein and Schreiber.[19] The rate of isomerization was thus estimated as 3.8% [0.55/(14.0 + 0.55)] of the total formolysis reaction of androsterone tosylate. Although this is clearly insufficient to represent the main reaction path, it would account for about 20% of the 2β-formoxy-5α-androstan-17-one (**6a**) that is formed by rearrangement. As there appears to be no simple and plausible pathway which would yield the 2α-tosylate (**7**) from the α-hydrogen-bridged intermediate (**3**), application of Winstein's proposal to the present case at most would explain only part of the rearrangement of androsterone tosylate.

Both mechanisms depicted in Scheme I involve a β-hydrogen-bridged species in the formation of the 2β-hydroxy compound and of one or the other isomer at C-3. It seemed that further information about the mechanism of the formolysis could be obtained if it were studied with androsterone stereospecifically labeled at C-2 with an isotope of hydrogen. Methods adaptable to the synthesis of both isomers have been given in reports on the preparation of 2α- and 2β-deuterio derivatives of 5α-cholestan-3α-ol. The former[20] was obtained by deuteroboration of 5α-cholest-2-ene followed by oxidation, the latter[20,21] by peroxidation of this olefin to the 2,3-α-epoxide and its reduction with lithium aluminum deuteride. The infrared spectra of the two products showed distinct C–D stretching bands[20] and, therefore, indicated sterically homogeneous preparations.[22]

The availability[8] of 17-ethylenedioxy-5α-androst-2-ene (**9**), free of its Δ^3 isomer, gave easy access to the 2,3-α-epoxide (**11**) by reaction with peroxybenzoic acid. This oxirane on treatment with partially tritiated lithium aluminum hydride and cleavage of the 17-acetal gave two major products: 86% of 3α- (**14a**) and 12% of 3β-hydroxy-5α-androstan-17-one (Scheme III). The formation of the latter cannot be ascribed to contamination of the epoxide with the 2,3-β isomer, because the known[8] product of its reaction with lithium aluminum hydride, the 2β-hydroxy-5α-androstan-17-one, was not found. Although such an inversion of the carbon atom that retains the oxygen apparently has not been reported with steroid epoxides, our observation need not occasion any surprise because analogous observations have been made with simpler epoxycyclohexanes.[23] Rickborn and Quartucci[23a] explained their

(19) S. Winstein and K. G. Schreiber, *J. Am. Chem. Soc.*, **74**, 2171 (1952).

(20) R. C. Cookson, D. P. G. Hamon, and R. E. Parker, *J. Chem. Soc.*, 5014 (1962).

(21) E. J. Corey, M. G. Howell, A. Boston, R. L. Young, and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 5036 (1956).

(22) See the mass spectrographic data on 2-labeled 5α-cholestan-6-one by C. Djerassi, R. H. Shapiro, and M. Vandewalle [*ibid.*, **87**, 4892 (1965)] for an independent confirmation of the stereospecificity of the procedure for the preparation of the 2β-deuterated isomer.

(23) (a) B. Rickborn and J. Quartucci, *J. Org. Chem.*, **29**, 3185 (1964); (b) N. A. LeBel and G. G. Ecke, *ibid.*, **30**, 4316 (1965).

TABLE III

ISOTOPE RATIOS AND DERIVED VALUES OF REACTION PRODUCTS[a]

	2α-T-A-3α-OTs (17b)				2β-T-A-3α-OTs (18b)			
	Formolysis				Formolysis			
		k_H/k_T		Addn,		k_H/k_T		Addn,
Compound	R	Mean	Range	R	R	Mean	Range	R
Starting	2.15			1.52	2.21			1.98
A-2β-OH	2.43	1.10	1.13–1.06	1.55	1.99	1.97	1.99–1.94	1.88
Oxidized	0.50			(0.12)	1.11			(0.30)
+Enolized	0.02				0.00			
A-3β-OH	2.93	1.00	±0.00		2.99	1.35	±0.00	
Oxidized	(2.73)				3.11			
A-3α-OH	2.31	0.95	1.04–0.69	1.62	1.97	1.82	1.90–1.55	1.90
Oxidized	2.13			1.53	1.78			1.77
A-2α-OH	(2.21)[b]			1.55	1.74			1.85
Olefin crude	1.52	1.46	1.46–1.47	1.52	1.98	1.99	±0.00	1.92
Olefin recrystd	(1.51)[c]				(1.91)[c]			

[a] Abbreviations as defined in legends to Table I. Formolysis refers to the two experiments that are averaged in run 3 of Table I; addn (additions) refers to those averaged in run 4 of Table I. Figures in parentheses refer to measurements for which constancy of isotope ratios within the limits stated in the Experimental Section could not be achieved. The isotope effects were calculated as described in the Appendix; the mean values are based on the average yields of the four runs of the addition experiment, the ranges on their lowest and highest values, respectively. *R*, ratio of $^3H_1/^{14}C_2$ counts as defined in the Experimental Section. [b] Isotope ratio rose during purification. [c] The mother liquors showed consistently higher isotope ratios than the crystals. Presumably, the former contained a somewhat higher proportion of the Δ^3 isomer.

finding by postulating hydrogen abstraction by AlH_3 from the oxygen-retaining carbon and subsequent reduction of the resulting ketone to the epimeric alcohol. The specific activity of our crude epiandrosterone was only 1.5 times as large as the one observed for androsterone rather than the nearly twofold value observed for the inverted alcohol in the reaction of *trans*-4-*t*-butyl-1,2-epoxycyclohexane with lithium aluminum deuteride.[23a] Since our conditions allowed for the operation of an isotope effect, it need not have been identical for the cleavage of the epoxide and for the subsequent reduction of the ketone. This mechanism might have allowed the introduction of ^3H at C-3 not only in epiandrosterone but to a lesser extent also in androsterone. Actually the amount of a doubly tritiated product in the latter preparation was negligible because androsterone-2β-^3H showed no significant loss of label on oxidation to the ketone.

Androsterone-2α-^3H (**10a**) was prepared simply by adapting the previously described[8] hydroboration reaction of 17-ethylenedioxy-5α-androst-2-ene (**9**) to a scale on which the tritiated reagent could be used. To allow for the convenient determination of specific activities of small samples of tritiated compounds with good precision, we mixed both preparations of tritiated androsterone (**10a** and **14a**) with a third species (**12a**) labeled with ^{14}C, since this in contrast to the tritiated forms could be expected to show no significant isotope effect. This androsterone-^{14}C was obtained from 3β-hydroxyandrost-5-en-17-one-4-^{14}C (**16**) by hydrogenation[24] of the 5,6 double bond and by inversion at C-3 by the method of Chang and Blickenstaff[25] [the reaction of the tosylate (**13**) with N,N-dimethylformamide] and hydrolysis of the resulting inverted formate. The isotopic purity of our preparations was demonstrated by obtaining consistent isotope ratios during the recrystallization of androsterone (**17a** and **18a**) and of two of its derivatives, the tosylate (**17b** and **18b**) and the diketone. While androsterone itself lost no tritium on enolization, all was removed on enolization of the diketone.

Both preparations of the doubly labeled androsterone tosylate (**17b** and **18b**) were subjected to formolysis under standard conditions, the formates were hydrolyzed, and the alcohols were separated and then oxidized. With one exception, all products (Table III) indicated some migration of tritium from C-2. This hydride shift was only minor[26] in the formation of the 3-formoxyandrostanones, but involved 65% of the 2β-tritium of androsterone tosylate in its conversion to the 2β-formoxy compound, if correction is made for the addition reaction. This value indicated and the results with the 2α-tritiated precursor confirmed that the migration of the 2β-hydrogen is not an obligatory step in the formation of the 2β-formoxy compound although it occurs along the major pathway to this product. When the 2,17-diketones were enolized all of the remaining tritium was lost, indicating that the oxidation had been complete, that none of the isotope was at C-4 and, therefore, that the olefin from which androsterone had been prepared was indeed free of the Δ^3 isomer as had been inferred from the spectral data.[8]

The over-all isotope effect of the reaction was deduced[29] (eq 1, Appendix) from the isotope ratio (Table IV) of a sample of androsterone tosylate isolated after an incomplete formolysis. As was to be anticipated from the work of Shiner and Jewett[30] with *cis*-4-*t*-butylcyclohexyl brosylates, the effect was considerably greater for the axially than for the equatorially placed isotope. When practically all of the labeled tosylate (isotope ratio R^0) is converted to products (isotope ratio R^i), the quotient (R^0/R^i) multiplied by the over-

(24) A. Butenandt, H. Dannenbaum, G. Hanisch, and H. Kudszus, *Z. Physiol. Chem.*, **237**, 57 (1935).

(25) F. C. Chang and R. T. Blickenstaff, *J. Am. Chem. Soc.*, **80**, 2906 (1958).

(26) The oxidation of alcohols in acetone with chromic acid–sulfuric acid reagent[27] admits the possibility of enolization. No loss of isotopic hydrogen from an adjacent carbon atom was reported for 17-hydroxy steroids[28] and no significant loss was observed on oxidizing **18a**. In spite of these vindications of the method, very small losses of tritium should probably be interpreted with caution and not be taken as definitive proof that the lost isotope had been attached to the carbon of a carbinol group.

(27) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(28) J. Fishman, *J. Am. Chem. Soc.*, **87**, 3455 (1965).

(29) J. Bigeleisen and M. Wolfsberg, *Advan. Chem. Phys.*, **1**, 38 (1958).

(30) V. J. Shiner, Jr., and J. G. Jewett, *J. Am. Chem. Soc.*, **86**, 945 (1964); **87**, 1382 (1965).

all isotope effect of the reaction equals the isotope effect of their formation (eq 9) provided that the reaction involves no loss of tritium. As this condition is not fulfilled for the olefin or the products derived from it, we have corrected for the addition reaction (eq 17) to estimate the isotope effects of the formolysis alone using the mean and extreme yields obtained in the addition experiments. The highest yield is regarded as a limiting value because it would account for the 2α-formate by addition alone. This, however, seems improbable because the 2α-hydroxy compound derived from the solvolysis of **17b** contained much more tritium than the one formed by addition to the olefin obtained from **17b**. Isotope effects can also be calculated according to eq 9 for the formation of the olefin fraction if its isotope ratio is corrected for the loss of tritium by elimination. This loss of isotope (Table IV) was surprisingly small (6%) in the case of the axially tritiated tosylate, but larger (23%) if the tritium was equatorial.

TABLE IV
ISOTOPE RATIOS AND DERIVED VALUES OF OVER-ALL REACTION[a]

	Ratio	2α-T-A-3α-OTs (**17b**)	2β-T-A-3α-OTs (**18b**)
Original A-3α-OTs	R[b]	2.15	2.21
A-3α-OTs, 2 hr	R	2.89	3.56
Crude product, 20 hr	R	1.66	2.08
Kinetic isotope effect	k_H/k_T	1.37	1.84
Elimination of T	% total	22.7	6.0
Elimination of T	% Δ[c]	25.0	6.6

[a] For abbreviations, see Table I. [b] R, ratio of $^3H_1/^{14}C_2$ counts as defined in the Experimental Section. [c] Percentage of olefin and olefin converted to other products.

The kinetic isotope effect for the formation of the 2β-formoxy-5α-androstan-17-one (**6a**) from the axially tritiated tosylate (**18b**) was strikingly higher than that for the formation of the 3β isomer. As the main reaction to the rearranged ester resulted in a migration of the isotopic hydrogen, it must involve a β-hydrogen-bridged structure either as a transition state or as an intermediate. The difference in the isotope effects is explicable, therefore, if the main path of the 3β isomer does not similarly require a direct participation of the carbon–tritium bond but proceeds through a classical C-3 cation. This conclusion is consistent with the mechanism proposed for the formation of the inverted ester from the *cis*-4-*t*-butylcyclohexyl tosylate by Winstein and Holness[13] rather than the one suggested by Cram and Tadanier[14] who derived this product mainly from a hydrogen-bridged intermediate.

These observations would be consistent with the solvolysis of the 3α-tosylate to the C-3 cation which then would be partitioned with different isotope effects into the 3β and the 2β esters. This simple scheme might also account for the high stereospecificity of the reaction at C-2 if it can be assumed that the tosylate ion remains on the α side of the steroid and forms an ion pair with the C-2 cation.[31] This postulate receives support from the isolation of the 2α-tosylate (**7**) since its formation would merely represent the combination of an ion pair. If the steric course of the reaction was to be attributed solely to the shielding effect of the departing tosylate ion, it seemed hard to explain why the substitution at C-2 showed much greater preference for β attack than at C-3, since β substitution at C-3 involves an equatorial approach whereas β substitution at C-2 requires the axial entry of the solvent in close proximity to the angular methyl group. A possible explanation for this apparent anomaly was provided by a referee who suggested that the C-3 ion pair might be less stable because the quasi-axial location of the anion might lead to greater steric interference than would be encountered by the anion in the quasi-equatorial location at C-2.

If in accordance with this scheme, the rate of formation of the C-3 ion determines the rate of formolysis of androsterone tosylate, its retardation by the 2β-tritium seems unusually large.[14,30,32] Moreover, in a related case, the solvolysis of *cis*-4-*t*-butylcyclohexyl brosylate in aqueous alcohol, Shiner and Jewett[30] observed that the isotope effects resulting from successive deuteriation of the two β-carbon atoms were not cumulative and deduced a direct participation of one of the axial C–D bonds in the ionization process. Finally, as relatively small inverse isotope effects are to be expected for the reaction of a classical carbonium ion with the solvent,[33] we anticipated a value even closer to 1 the ratio of isotope effects for a pair of epimers formed from the same classical ion simply by solvent attack from opposing directions. However, regardless of the estimate made of the contribution of the addition reaction, a marked difference was encountered in the effects of tritium on the formation of the 3α and 3β esters from either one or the other tosylate (**17b** or **18b**).

There seems to be some justification, therefore, to consider tentatively also a second reaction mechanism with two competing transition states for the formolysis of androsterone tosylate, one leading to the C-3 cation and another characterized by a larger kinetic effect of 2β-tritium. As the reciprocal of the observed isotope effects of a solvolysis with two primary products is the weighted mean of the reciprocals for the two ionization products (eq 18), the second transition state would be characterized by a 2β-tritium effect larger than 1.84. Such a value would seem appropriate if the second ionic species that is formed directly from the 3α-tosylate would be either the C-2 classical ion that results from the shift of the 2β-hydrogen or a β-hydrogen-bridged structure. If one considers the yield of and the isotope effect on the 2-β-formate by means of eq 18, it is apparent that this ester cannot be the sole product of its ionic precursor if the 2β-tritium effect on the formation of the C-3 classical ion is to have a value within the usual range. On this basis a major portion of the only quantitatively important product, the olefin, would also

(31) It can be questioned whether the close association of the ion pair can be maintained during the migration of the 2α hydrogen. This lesser route to the 2β-formate, however, presents no special stereochemical problem since the reaction at C-2 is an inversion.

(32) According to the equation given by C. G. Swain, E. C. Stivers, J. F. Reuwer, Jr., and L. J. Schaad [*J. Am. Chem. Soc.*, **80**, 5885 (1958)] and by L. Melander ("Isotope Effects on Reaction Rates," The Ronald Press Co., New York, N. Y., 1960, p 24) the kinetic isotope effects k_H/k_D for the formolysis of androsterone tosylate can be estimated from measurements on the tritiated species to be 1.53 if the isotope is axial, and 1.24 if equatorial at C-2 in the starting compound.

(33) P. Laszlo and Z. Welvart, *Bull. Soc. Chim. France*, 2412 (1966); G. S. Hammond, *J. Am. Chem. Soc.*, **77**, 334 (1955).

have to be derived from a precursor other than the C-3 classical ion.[34]

This makes it unlikely that the second ionic species directly derived from the 3α-tosylate is the C-2 cation tosylate anion pair. One would expect that this ion pair would yield about the same ratio of products whether generated from the 2α- or from the 3α-tosylate. If on this basis one compares the yields of the 2β-formate from the 3α- and 2α-tosylates, the olefin fraction resulting from the former *via* the C-2 ion pair approximates only 15%. Moreover, only the 2α-tosylate gave easily detectable amounts of a product with the spectral characteristics of a Δ^1-olefin.[35a] Therefore, it is more likely that the sought for second major source of the olefin (beside the C-3 ion)[34] is the β-hydrogen-bridged ion pair. If this ion pair dissociates, the usual diaxial opening of a three-membered ring can occur and the 3α ester would result. If the bridged ion remains paired with the tosylate, it should yield preferentially the C-2 rather than the C-3 cation pair since the latter has an additional interaction of an axial hydrogen (at C-2) with an angular methyl group. The subsequent reactions of the C-2 ion pair to the 2β-formate, to the 2α-tosylate, and to some olefin have already been discussed. It seems possible therefore to account for the steric course of the rearrangement also within the general framework of the Winstein–Holness hypothesis, but without the need for postulating another hydrogen-bridged structure (**3**) or the opening of a bridge with retention of configuration. Although the available data on isotope effects do not afford an adequate test of this mechanism, further clarification may result from a study of the solvolysis of androsterone tosylate tritiated at C-4 and from a more reliable[35b] comparison of the effect of 2β-tritium on the formation of 2β and 3α esters.

The losses of isotope by elimination provide information about the stereochemistry of this process in the unlabeled compound only if one knows the isotope effects. If one assumes, for lack of information, that the ratio of the rate of detaching the 2α-hydrogen over that of removing the 2α-tritium was equal[36] to the ratio of the corresponding rates for the 2β bond, the predominance of the loss of the equatorial over the axial hydrogen in the unlabeled compound (eq 23) would be 5:1. This steric preference is similar to one observed by Cookson, *et al.*,[20] in the acid-catalyzed conversion in aqueous dioxane of 3-methylene-5α-cholestane into 3-methyl-5α-cholest-2-ene, if their results are computed in the same manner.[37] Although it is quite possible that *cis* elimination is less strongly preferred than this simplified analysis of our data would indicate, it seems substantial enough to constitute a notable exception to the generalization[38] that the diaxial mode of elimination is the preferred pathway in E1 reactions. A possible explanation may lie in the steric hindrance exerted by the angular methyl group toward solvation from the β side. This might allow an unusually large role for attack by the departing tosylate on the 2α-hydrogen.

The stereochemistry of the addition reaction to the Δ^2-5α-olefin also has an unusual aspect. Those studied before have demonstrated steric hindrance to the addition from the β side. This is shown by the *cis*-addition reactions with peroxy acids,[39] diborane,[8] or osmium tetroxide[40] which gave mainly or exclusively α derivatives. In ionic additions (hypobromous acid,[40] chromyl chloride,[40] iodine trifluoroacetate[41]) the cation added α (axially) at C-3 and the nucleophilic agent axially at C-2 (β). The additions of formic and trifluoroacetic[42] acid do not conform to this pattern. Although the axial addition of the nucleophilic agent was preserved, the predominant entry was now at C-3, particularly when trifluoroacetic acid was used. As pointed out by Collins and Hammond[43a] the mechanism proposed for the addition of hydrogen halides which postulates as the rate-determining step the conversion of a π complex to a carbonium ion does not explain a highly stereospecific entry of the nucleophilic addendum. Two mechanisms would avoid this difficulty. Winstein and Holness[13] postulated hydrogen-bridged intermediates in the formation of the axial esters which they obtained on adding formic acid to 4-*t*-butylcyclohexene. In the absence of a shielding counterion, these intermediates might well behave unlike the corresponding structures generated from the tosylate. In our case we would expect that the 2α,3α-hydrogen-bridged cation would form much more rapidly, but that the 2β,3β isomer would be the one that is more reactive toward the opening of the ring. In this situation the 3α ester could be the preferred product if the opening of the α-bridged ion was slower than its return to the olefin. The alternative explanation[43b] is a concerted process which would have the stereospecificity observed on the assumption that the space requirements are more stringent for the closer approach of the nucleophilic agent than of the proton donor. With either mechanism the increased selectivity observed with trifluoroacetic acid as compared to formic acid could be ascribed to its greater bulk.

Experimental Section

General Procedures.—Melting points are corrected. Infrared spectra were measured on solutions in carbon disulfide with a double-beam grating spectrophotometer (Perkin-Elmer, Model 421).

(34) The lack of an effect of 2α-tritium on the rate of formation of the 3β ester from **17b** suggests that this formate is formed from the C-3 cation in competition with a process having a larger isotope effect, presumably the elimination of the 2α-tritium. It appears therefore that the C-3 cation is also a source of olefin.

(35) (a) G. M. L. Cragg, C. W. Davey, D. N. Hall, G. D. Meakins, E. E. Richards, and T. L. Whateley, *J. Chem. Soc., C*, 1266 (1966). (b) This requires either a solvent system in which the addition reaction is suppressed or one (*e.g.*, HCOOD) which allows one to measure directly the contribution of the addition reaction to the products formed by substitution of the tosylate.

(36) This simplifying assumption is analogous to one first proposed by D. Y. Curtin and D. B. Kellom [*J. Am. Chem. Soc.*, **75**, 6011 (1953)] for an aliphatic system.

(37) Cookson, *et al.*,[20] have used the equations of Curtin and Kellom[36] which, however, are inapplicable to our case because the formation of the Δ^3-olefin[3] represents additional competing reactions to the *cis* and *trans* eliminations from C-2. It should be further noted that the significance of the steric and isotopic factors in the treatment by Curtin and Kellom differ if the discrimination between the isotopes occurs during the rate-determining step or follows it. In the *absence* of secondary effects the rate of removal of hydrogen from the labeled molecule equals that of its removal from the unlabeled molecule only in the first case. In the second there would be compensatory accelerations which would not cancel as they differ greatly for the major and the minor pathways.

(38) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(39) A. Fürst and P. A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949).

(40) H. L. Slates and N. L. Wendler, *J. Am. Chem. Soc.*, **78**, 3749 (1956).

(41) D. G. Hey, G. D. Meakins, and M. W. Pemberton, *J. Chem. Soc., Sect. C*, 1331 (1966).

(42) P. E. Peterson, *J. Am. Chem. Soc.*, **82**, 5834 (1960).

(43) (a) C. H. Collins and G. S. Hammond, *J. Org. Chem.*, **25**, 911 (1960); (b) G. S. Hammond and C. H. Collins, *J. Am. Chem. Soc.*, **82**, 4323 (1960).

All separations of unesterified 17-oxo steroids were carried out by chromatography on alumina (Woelm, nearly neutral, deactivated with 7% water). The ratio of adsorbent to the anticipated weight of the steroid alcohols was 1000 or more. In the usual column (13 × 200 mm, 24 g) the mixing chamber for the gradient elution contained 800 ml of benzene which was being replaced at a volume ratio of 13:1 by benzene containing 5% ethanol and the eluate was collected in 7-ml fractions. This procedure gave complete separations of all identified reaction products except 2β- and 3β-hydroxy-5α-androstan-17-one. These were then separated by chromatography on 0.5-mm layers of silica gel–calcium sulfate (Adsorbosil 1 of Applied Science Laboratories, State College, Pa.) in benzene containing 6% of ethanol. In runs with radioactive material, the thin layer chromatography was repeated with each fraction after diluting it with the unlabeled isomer that was to be removed.[44]

Unless noted otherwise, the formolyses of androsterone tosylate and the addition reactions of formic acid to androstenone that are reported in Tables I to IV were conducted as follows. The steroid was dissolved in benzene and acetone (4.6 and 6.8 ml per liter of final solution) and made 0.0035 *M* by the addition of formic acid which had been dried over anhydrous copper(II) sulfate and distilled *in vacuo*. The reaction was terminated by distributing the solution between four volumes of benzene and three volumes of water. The organic phase was washed repeatedly with 5% sodium carbonate and with water and taken to dryness under reduced pressure. If the reaction was allowed to go to completion, formates were hydrolyzed by heating a solution in methanol and 5% aqueous potassium bicarbonate (5:1, v/v) under a reflux for 90 min. The mixture was distributed between benzene and water and the washed benzene solution was taken to dryness *in vacuo* and chromatographed on alumina as described above. When the reaction was not allowed to go to completion, the hydrolysis of the formates was omitted and the tosylates were isolated by partition chromatography on washed cellulose (2000 times the weight of total steroids) that contained per gram 0.5 ml of the polar phase of the system ligroin methanol–water (10:9:1). Development of this column with the lighter phase of this system eluted the olefin and the formates ahead of the tosylate fraction.

Kinetic Measurements.—The amount of tosylate remaining in the reaction mixture was determined by ultraviolet spectrophotometry (Beckman Model DU with photomultiplier) on the residue of the neutral benzene extract of the reaction mixture in the manner previously described.[17] The correction for nonspecific absorption was based on measurements taken after formolyses for 23 and 30 hr, respectively, of 3α- and 2α-tosyloxy-5α-androstan-17-one. The size of this correction became large after 6 half-lives. If the ratio of the two tosylates had to be determined, these were purified by partition chromatography as described above and examined at 934, 903, and 573 cm^{-1}. The isomer ratio was taken as the mean of those calculated from the absorbance at 903 and either that at 934 or at 573 cm^{-1}. This mean differed from the individual values by 1–2%.

Radioactivity Measurements.—These were done by scintillation counting with a two-channel spectrometer (Tricarb of Packard Instrument Co., Model 314EX) on solutions in 5 ml of toluene containing 20 mg of 2,5-diphenyloxazole and 0.5 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene. The isotope ratio $^3H_1/^{14}C_2$ refers to the tritium counts in the first channel to the ^{14}C counts in the second. It was calculated as previously described.[45] The $^{14}C_2$ counts were calculated according to Okita, *et al.*[46] The usual ratios, N_2/N_1, of counts registered in the two channels for a single isotope were 0.01 for ^3H and 8.6 for ^{14}C. Compounds were purified by recrystallization until the isotope ratio of two successive mother liquors agreed within 3%. At this stage the ratio for crystals and mother liquor also usually agreed within the same limits. Exceptions are indicated in Table III. The amount of radioactive 2α-hydroxy-5α-androstan-17-one was insufficient for purification by recrystallization. Constancy of isotope ratios was tested here after thin layer and paper chromatography. When the over-all isotope effect of the reaction was measured, the tosylate recovered after 2 hr was isolated by partition chromatography and recrystallized from benzene–petroleum ether (bp 60–70°) until the mother liquor was free of 2α-tosyloxy-5α-androstan-17-one in spectrographically detectable amounts. To locate tritium, the hydroxy ketones were oxidized in acetone with 8 *N* chromium trioxide–sulfuric acid[47] at 15° for 10 min and the neutral reaction products were recrystallized. The diketones in 5 ml of 80% ethanol containing 32 mg of sodium hydroxide were heated under a reflux for 4 hr and were again isolated by ether extraction.

As far as possible, the final measurements used to calculate the tabulated isotope ratios were done simultaneously for any experiment and continued until at least 50,000 net counts had registered. In a few cases where simultaneous assay was not feasible, correction for variations in spectrometer efficiency was made.

Preparation of Tosylates.—These were obtained from the parent alcohols[8] by reaction with *p*-toluenesulfonyl chloride–pyridine (1:4) at room temperature for 24 hr. Water was added to hydrolyze the excess reagent and the product was isolated by distributing the mixture between benzene and water, by washing the benzene phase with hydrochloric acid, sodium carbonate solution, and with water, and by distillation of the solvent *in vacuo*. The infrared spectra of the recrystallized products showed the five bands common to steroid tosylates[17] and no hydroxyl absorption.

Androsterone tosylate (1) was recrystallized from acetone and had mp 159–160° dec (lit.[9] mp 149–150°). The spectrum is given in Figure 1.

3β-Tosyloxy-5α-androstan-17-one was recrystallized from benzene–petroleum ether and from acetone and had mp 161–163° dec (lit. mp 163–164°[47a] and 164–165°[9]). Prominent tosylate bands characteristic of this isomer were at 946, 937, 902, 864, 848, 813, 668, and 564 cm^{-1}.

2α-Tosyloxy-5α-androstan-17-one (7) was recrystallized from benzene–petroleum ether and had mp 126–127.5°. For the spectrum, see Figure 1.

2β-Tosyloxy-5α-androstan-17-one was recrystallized from benzene–petroleum ether and from acetone–petroleum ether and had mp 132–134° dec. Prominent tosylate bands characteristic of this isomer were at 925, 911, 893, 878, 813, and 686 cm^{-1}.

Identification of Formolysis Products.—These three experiments (A–C) were done with commercial formic acid (97–100%) without further drying. No cosolvents were used. The concentration of the tosylate was about 0.0005 *M*.

A. Androsterone Tosylate (1) (Complete Reaction).—After keeping a solution of 370.6 mg of androsterone tosylate at 25° for 17 hr, sodium formate (1 g) was added and the solution was concentrated *in vacuo* at a bath temperature of 25°. The neutral reaction product was obtained by distribution between benzene and water and the usual washing. It was hydrolyzed with bicarbonate as described above and chromatographed on alumina. A mixture of the Δ^2 and Δ^3 isomers of 5α-androsten-17-one (estimated[8] to be 9:1) and 2α- (**8**), 2β- (**6b**), 3α- (**5b**), and 3β-hydroxy-5α-androstan-17-one (**4b**) were identified by their infrared spectra, which were in good agreement with those of reference compounds.[8] The 2α isomer was further characterized by the infrared spectrum of its acetate, the three other alcohols were identified by their melting points.[47b] The yields are given in Table I, run 2.

B. Androsterone Tosylate (1) (Incomplete Reaction). Isolation of 2α-Tosyloxy-5α-androstan-17-one (7).—A total of 494 mg of androsterone tosylate in three batches was kept in formic acid at 1–7° for 48–72 hr. Each batch after the addition of 530 mg of sodium formate was concentrated under reduced pressure and distributed between benzene and water. The residue of each organic phase which had been washed with bicarbonate and water, was recrystallized from a mixture of benzene and petroleum ether and gave unchanged starting material. The combined mother liquors (288 mg) were fractionated by partition chromatography as described above. The tosylate fraction (15.8 mg) was recrystallized from methanol–acetone. The 2α-tosyloxy-

(44) This washing out of the labeled impurity allowed the use of the whole chromatographic bands of the isomeric alcohols. This was essential because the tritiated compounds, in accord with observations of P. D. Klein, D. W. Simborg, and P. A. Szczepanik [*Pure Appl. Chem.*, **8**, 357 (1964)] and others, moved at rates slightly different from those of their ^{14}C-labeled analogs. If only a portion of a band had been used, the sampling would not have been representative.

(45) R. G. Wieland, C. de Courcy, R. P. Levy, A. P. Zala, and H. Hirschmann, *J. Clin. Invest.*, **44**, 159 (1965).

(46) G. T. Okita, J. J. Kabara, F. Richardson, and G. V. LeRoy, *Nucleonics*, **15** (6), 111 (1957).

(47) (a) J. Iriarte, G. Rosenkranz, and F. Sondheimer, *J. Org. Chem.*, **20**, 542 (1955). (b) A more comprehensive identification of these products was carried out when they were first obtained by hydrolysis of androsterone sulfate.[8]

5α-androstan-17-one (**7**) was concentrated in the mother liquor (9.1 mg) and was isolated (3.9 mg) from this fraction by crystallization from benzene–petroleum ether. It had mp 123–126°, and 122.5–125.5° after admixture with a reference sample prepared from 2α-hydroxy-5α-androstan-17-one (**8**).[8] The infrared spectra are compared in Figure 1.

C. 2α-Tosyloxy-5α-androstan-17-one (7).—A solution of 27.7 mg of this compound in 120 ml of formic acid was kept at 25° for 17 hr. The product was isolated, hydrolyzed, and chromatographed as described for the standard procedure and gave 13.1 mg of an olefin and 3.7 mg of 2β-hydroxy-5α-androstan-17-one (**6b**) and traces of spectrographically not quite pure 2α-, 3α-, and 3β-hydroxy-5α-androstan-17-one. The spectrum of the olefin showed it to be a mixture of 5α-androst-2-en-17-one and a contaminant with absorption peaks close to those reported[35a] for 5α-cholest-1-ene. Compound **6b** was identified by its infrared spectrum and its melting point (191–193°).

Androsterone-4-^{14}C (12a).—3β-Hydroxyandrost-5-en-17-one-4-^{14}C (**16**), from New England Nuclear Corp., was diluted with cold carrier and recrystallized to constant specific activity. A solution (32.6 mg, about 10 μcuries) in 10 ml of 95% ethanol was shaken with 280 mg of freshly reduced palladium–calcium carbonate[24] (1%) for 1 hr. The product (**15**) was shown to be free of starting material by paper chromatography with ligroin–methanol–water (10:9:1).[48] It was converted to 3β-tosyloxy-5α-androstan-17-one (**13**) as described above. After three recrystallizations the last mother liquor had the same infrared spectrum as the reference compound. The crystals (37 mg) in 6 ml of dimethylformamide and 0.2 ml of water were heated under a reflux for 66 hr. The resulting mixture of olefin and formate was hydrolyzed with potassium bicarbonate in boiling methanol for 1.5 hr. The resulting material (24.3 mg) was adsorbed on 25 g of alumina. Elution with benzene–petroleum ether gave 4.7 mg of olefin and with benzene containing 2% ethanol 19.4 mg of androsterone (**12a**). After four recrystallizations from dilute methanol it had mp 182.5–184°. The infrared spectrum of the second mother liquor showed no impurity.

Androsterone-2α-^{3}H (10a).—Tritiated diborane was generated by adding a mixture of 10.5 mg of tritiated sodium borohydride (29 mcuries, New England Nuclear Corp.) and 41 mg of ordinary sodium borohydride in 2 ml of diglyme to 0.5 ml of boron trifluoride etherate in 2 ml of diglyme, and passed into a solution of 16 mg of 17-ethylenedioxy-5α-androst-2-ene (**9**) (free of the Δ^3 isomer) in 2 ml of tetrahydrofuran. The solvents had been freshly purified. The apparatus differed from that described by Brown and Subba Rao[49] in having smaller dimensions, ground-glass connections, and no mercury safety release valve. The reaction was allowed to proceed for 3 hr. Subsequent steps were carried out as described[8] for the preparation of 2α-hydroxy-5α-androstan-17-one. The mixture of 17-ketones was heated for 2 hr in 5 ml of 80% ethanol containing 32 mg of sodium hydroxide to remove any dissociable tritium that might be present, and the resulting material was fractionated by gradient elution from alumina as described above. The resulting androsterone from the central part of the peak was recrystallized from dilute methanol. The crystals (**10a**, 3.8 mg) had mp 183–184.5°. The infrared spectrum of the combined first and second mother liquors showed no impurities. A portion of the product (0.84 mg) was combined with cold carrier (515 mg) and with androsterone-4-^{14}C (**12a**, 4.1 mg) and recrystallized three times. The following isotope ratios $^{3}H_1/^{14}C_2$ were observed: the last two mother liquors of this preparation (**17a**), 2.16 and 2.19; the final crystals, 2.18; the toluenesulfonate (prepared as described above and recrystallized) (**17b**), 2.15; 5α-androstan-3,17-dione (recrystallized), 2.09 (0.02 after enolization).

2α,3α-Epoxy-17-ethylenedioxy-5α-androstane (11).—17-Ethylenedioxy-5α-androst-2-ene (**9**) (43.5 mg, 0.14 mmole, free of the bond isomer) and 0.68 mmole of peroxybenzoic acid in 3.8 ml of benzene and 0.2 ml of petroleum ether were kept at 7° for 6 hr. The neutral product was recrystallized from methanol to give the oxirane **11**, mp 143.5–145.5°. The infrared spectrum showed no carbonyl or olefin absorption. A strong peak at 802 cm^{-1} coincides with one reported[35a] for 2α,3α-epoxy-5α-cholestane.

Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found:[50] C, 75.79; H, 9.51.

Androsterone-2β-^{3}H (14a).—A slurry of 25 mg of ordinary lithium aluminum hydride and of 2 mg of a tritiated sample (5 mcuries) in 8 ml of ether was added to 11.3 mg of 2α,3α-epoxy-17-ethylenedioxy-5α-androstane and heated under a reflux for 2.5 hr. The excess of hydride was decomposed by adding 1 ml of 95% ethanol. The product was kept in 5 ml of ethanol and 2.6 ml of 0.5 *N* hydrochloric acid for 2.5 hr and the resulting ketone was heated like the 2-epimer with alkali to remove any dissociable tritium. The final product (9.4 mg) was fractionated by gradient elution from 24 g of alumina. Three peaks of radioactivity were observed which contained 18, 80, and 2% of the recovered radioactivity. The center portion of the main peak was recrystallized twice from dilute methanol. The first mother liquor showed the infrared spectrum of androsterone and the crystals had mp 183.5–185°. After the addition of 527 mg of carrier and 4.1 mg of androsterone-4-^{14}C (**12a**) to 0.24 mg of this product (**14a**), the mixture (**18a**) was recrystallized three times. The isotope ratios were as follows: the last two mother liquors, 2.16 and 2.16; the final crystals, 2.20; the toluenesulfonate after recrystallization, 2.21; 5α-androstane-3,17-dione (recrystallized), 2.18 (0.00 after enolization).

The first peak of the chromatogram showed the infrared spectrum of 3β-hydroxy-5α-androstan-17-one (**4**). The specific activity of the crude preparation was 1.51 times that of the tritiated androsterone-2β-^{3}H (**14a**) (before the addition of carrier and of the ^{14}C-tracer). The material of the last peak was not identified. Its mobility on the column suggested identity with 2α-hydroxy-5α-androstan-17-one (**8**).

Reaction of 5α-Androstan-17-one with Trifluoroacetic Acid.—The olefin (100.6 mg) that results from the formolysis of androsterone tosylate was dissolved in 20 ml of redistilled trifluoroacetic acid and kept at 25° for 2 days. The mixture was distributed between ether and water. The ether phase was washed with sodium carbonate and water and taken to dryness. The product, and 64 mg of sodium hydroxide were heated under a reflux in 10 ml of 80% ethanol for 15 min. The neutral reaction product was chromatographed on alumina with gradient elution. The spectrum of the early eluates (21.4 mg of an oil) showed that there could be only little of the starting material. A new rather weak peak in the region of olefinic C–H stretching had appeared. This material was followed by three unidentified hydroxy 17-ketones in small amounts and then by the four 2- and 3-hydroxy-5α-androstan-17-ones. These were identified by their infrared spectra and the 2α, 2β, and 3α isomers by their melting points after recrystallization. The yields are given in Table I, run 5.

Registry No.—**1**, 10429-00-2; **6b**, 10429-01-3; **7**, 10429-02-4; **10a**, 10429-03-5; **11**, 10429-04-6; **12a**, 10415-45-9; **14a**, 10429-05-7; **17b**, 10429-06-8; **18b**, 10415-46-0; 3β-tosyloxy-5α-androstan-17-one, 10429-07-9.

Appendix

Definitions.—*k* is the reaction rate; *s* is the specific activity; *R* is the isotope ratio ($^{3}H_1/^{14}C_2$ as defined in the Experimental Section); *n* is the fraction of reaction products that is found in a given product; *m* is the fractional yield from olefin in 20 hr. These terms are modified by superscripts 1, 2...*i* to indicate the first, second.....*i*th reaction product. The superscripts 0 and *t* refer to measurements of androsterone tosylate at the start of the reaction and after the time *t*; Δ refers to total olefin; F refers to the direct pathway of formolysis; and A refers to the route by addition to the olefin. Subscripts T, 2α-T, 2β-T, C, and H are used to indicate labeling with tritium, with tritium initially at the 2α or 2β position and with ^{14}C, and no labeling, respectively. The term *a* refers to the initial concentration of androsterone tosylate, *x* to the concentration of the products derived from it after the time *t*, and *b*

(48) For a closely related solvent system for the separation of **15** and **16**, see K. D. Roberts, R. L. VandeWiele, and S. Lieberman, *J. Biol. Chem.*, **236**, 2213 (1961).

(49) H. C. Brown and B. C. Subba Rao, *J. Am. Chem. Soc.*, **81**, 6428 (1959).

(50) Analysis was by Mr. A. W. Spang, Ann Arbor, Mich.

to the total amount per unit volume, of olefin produced in the reaction.

Isotope Effect of Over-all Reactions.—A derivation analogous to the one given by Bigeleisen and Wolfsberg[29] yields

$$k_H/k_T = \log [a/(a - x)]/\log [a/(a - x)] - \log (R^t/R^0) \quad (1)$$

Isotope Effect in Formation of Individual Products (k_H^i/k_T^i).

$$k_C = k_C^1 + k_C^2 + \ldots. \quad (2)$$

$$k_T = k_T^1 + k_T^2 + \ldots. \quad (3)$$

By definition of n

$$k_C^i = n_C^i k_C \quad (4)$$

and

$$k_T^i = n_T^i k_T. \quad (5)$$

Dividing (4) by (5)

$$k_C^i/k_{\perp}^i = (n_C^i/n_T^i)(k_C/k_T) \quad (6)$$

After the reactions for both isotopes have gone practically to completion and if product i has lost no tritium by elimination

$$R^i = as_T^0 n_T^i/as_C^0 n_C^i = R^0 n_T^i/n_C^i \quad (7)$$

Substituting (7) in (6)

$$k_C^i/k_T^i = (R^0/R^i)(k_C/k_T) \quad (8)$$

and since $k_C = k_H$ and $k_C^i = k_H^i$

$$k_H^i/k_T^i = (R^0/R^i)(k_H/k_T) \quad (9)$$

Correction for Addition Reaction.

$$x = a - ae^{-kt} \quad (10)$$

$$\int x dt = \int (a - ae^{-kt}) dt = at + (a/k)e^{-kt} \quad (11)$$

$$\int_0^t x dt = at - (a/k)(1 - e^{-kt}) \quad (12)$$

At prevailing values of t (20 hr) and of rates, this can be simplified to

$$\int_0^t x dt = at - a/k \quad (13)$$

Therefore, the fraction f of total olefin formed by formolysis from a, that is available for addition reaction during the time t is

$$f = (an^{\Delta}t - an^{\Delta}/k)/an^{\Delta}t = 1 - 1/kt \quad (14)$$

Correction of Isotope Ratios R^i for Addition Reactions.

$$R^i = (as_T^0 n_T^{iF} + bf_T s_T^{\Delta} m_T^{iA})/(as_C^0 n_C^{iF} + bf_C s_C^{\Delta} m_C^{iA}) \quad (15)$$

Since $s_T^0/s_C^0 = R^0$, $s_C^{\Delta} = s_C^0$, $s_T^{\Delta}/s_C^{\Delta} = R^{\Delta}$, $f_C = f_H$, $m_C^{iA} = m_H^{iA}$, and $n_C^{iF} = n_H^{iF}$, eq 15 can be rewritten as

$$n_T^{iF}/n_C^{iF} = (R^i/R^0)\{1 + (b/a)(m_H^{iA}/n_H^{iF})[f_H - f_T(R^{\Delta}/R^i)(m_T^{iA}/m_H^{iF})]\} \quad (16)$$

Although the ratio R^{Δ}/R^{iA} is not the same after 20 and 163 hr, the isotope effect of the addition reaction is so small that this change with time which can be calculated from the data of Collins and Lietzke[51] can be ignored. Therefore, $R^{iA} = R^{\Delta}m_T^{iA}/m_H^{iA}$ and

$$R^{iF} = R^0 n_T^{iF}/n_C^{iF} = R^i[1 + (b/a)(m_H^{iA}/n_H^{iF})(f_H - f_T R^{iA}/R^i)] \quad (17)$$

gives the corrected isotope ratio to be substituted in (9). The f values needed to solve (17) were calculated from (14).

Interrelationship of Isotope Effects.—From (3)

$$k_T/k_H = k_T^1/k_H + k_T^2/k_H^+ \ldots \quad (18)$$
$$= (k_T^1/k_H^1)(k_H^1/k_H) + (k_T^2/k_H^2)(k_H^2/k_H) + \ldots$$
$$= (k_T^1/k_H^1)n_H^1 + (k_T^2/k_H^2)n_H^2 + \ldots$$

If the fractional yields n are known for the reaction with the tritiated material, by analogous derivation for a product i that is formed by two or more pathways with the rates $k^{i(1)}$, $k^{i(2)}$,. . . ., one obtains

$$k_H^i/k_T^i = (k_H^{i(1)}/k_T^{i(1)})n_T^{i(1)} + (k_H^{i(2)}/k_T^{i(2)})n_T^{i(2)} + \ldots \quad (19)$$

Ratio of *cis* Elimination (Removal of 2α-Hydrogen) to *trans* Elimination (Removal of 2β-Hydrogen).

$$\text{Assumption: } k_H^{cis}/k_{2\alpha\text{-}T}^{cis} = k_H^{trans}/k_{2\beta\text{-}T}^{trans} \quad (20)$$

$$k_{2\beta\text{-}T}^{trans} = gk_{2\beta\text{-}T}^{\Delta} \quad (21)$$

$$k_{2\alpha\text{-}T}^{cis} = hk_{2\alpha\text{-}T}^{\Delta} \quad (22)$$

where g and h refer to the fraction of olefin that lost tritium. Substituting (21) and (22) in (20)

$$k_H^{trans}/k_H^{cis} = gk_{2\beta\text{-}T}^{\Delta}/hk_{2\alpha\text{-}T}^{\Delta} = (g/h)(k_{2\beta\text{-}T}^{\Delta}/k_H^{\Delta})(k_H^{\Delta}/k_{2\alpha\text{-}T}^{\Delta}) \quad (23)$$

The two isotope effects which appear in the last term can be calculated by eq 9 if the tritium count of the olefin is corrected for the fraction of tritium lost by elimination.

(51) C. J. Collins and M. H. Lietzke, *J. Am. Chem. Soc.*, **81**, 5379 (1959).