These data reveal that the isotope effects due to *equatorial* (*trans* to the leaving group) deuterium substitution are not only larger, but they are very nearly cumulative ($\sqrt{2.087/1.127} = 1.361$); that is, the two equatorial β-hydrogen atoms seem to be *equivalent* in the solvolytic transition state. The slight discrepancy may be due to loss of deuterium in the epimerization reaction. On the other hand, the two *axial* (*cis* to the leaving group) β-hydrogen atoms seem to be *nonequivalent* in the transition state; the rate retardation caused by 2,6-diaxial deuteration exceeds the square of 1.127 (the effect of the second axial β-deuterium = $2.425/2.087 = 1.164$).

The effect of each β-deuterium atom in the solvolytic transition state may be calculated as was previously described.[1] Such an analysis (assuming exactly cumulative equatorial behavior) leads to the distribution shown in partial structure II. The striking conforma-

II
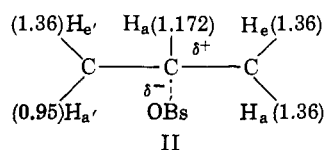
tional dependence of the β-deuterium isotope effect in this system can be explained by a solvolytic transition state which involves a nonchair (*e.g.*, twist boat)[5] conformation. The slight deviation from cumulative behavior for equatorial β-deuterium substitution can also be accommodated in terms of a twist-boat-like conformation for the activated state (*i.e.*, the *degree* of "twist" may determine this). It is difficult to see how these isotope effects and product data can be in accord with a chair conformation of the ring in the solvolytic transition state.

Acknowledgment. The authors gratefully acknowledge partial support of this work by Grant No. AT11-1-1008 of the U. S. Atomic Energy Commission and the use of the facilities of the Indiana University Research Computing Center.

(5) J. B. Hendrickson, *J. Am. Chem. Soc.*, 83, 4537 (1961).

V. J. Shiner, Jr., John G. Jewett

Department of Chemistry, Indiana University
Bloomington, Indiana
Received November 13, 1964

The Transition State in Acetolysis of Cyclohexyl Tosylate

Sir:

Some time ago we studied the acetolysis of deuterated cyclohexyl tosylates, but delayed publication because insufficient information was available to permit interpretation of our data. The results of Shiner and Jewett[1,2] point to a persuasive explanation and move us to report our work at this time.

The following deuterated cyclohexanols were prepared: 1-*d*, 2,2,6,6-*d*₄, *trans*-2-*d*, and *cis*-2-*d*. All except the last of these were obtained by the procedures used by Streitwieser[3] for the analogous cyclopentanols.

(1) V. J. Shiner, Jr., and J. G. Jewett, *J. Am. Chem. Soc.*, 87, 1382 (1965).
(2) V. J. Shiner, Jr., and J. G. Jewett, *ibid.*, 87, 1383 (1965).
(3) A. Streitwieser, Jr., R. H. Jagow, R. C. Fahey, and S. Suzuki, *ibid.*, 80, 2326 (1958).

Table I. Rates of Acetolysis of Deuterated Cyclohexyl Tosylates at 50.00°[a]

Compd.	$k_1 \times 10^6$, sec.$^{-1}$ [b]	k_H/k_D
H	2.37 ± 0.02	
1-*d*	1.95 ± 0.02	1.22[c]
H	2.38 ± 0.02	
cis-2-*d*	1.91 ± 0.02	1.25
H	2.36 ± 0.02	
trans-2-*d*	1.82 ± 0.03	1.30
H	2.39 ± 0.04	
2,2,6,6-*d*₄	1.02 ± 0.03	2.34

[a] Determined in 0.05 M solutions in anhydrous acetic acid containing 0.08 M sodium acetate. Aliquots were quenched in glacial acetic acid and titrated with 0.05 M perchloric acid in glacial acetic acid to the crystal violet end point. Temperature control was good to 0.02°. [b] Parallel runs on deuterated and undeuterated compounds in each case. Rate constants are averages of duplicate determinations with indicated deviations from the averages. [c] A value of 1.19 at 75.4° is reported by K. Mislow, S. Borčić, and V. Prelog, *Helv. Chim. Acta*, 40, 2477 (1957).

cis-Cyclohexanol-2-*d* resulted from deuterioboration of cyclohexene followed by oxidation with alkaline hydrogen- peroxide.[4] The tosylates of the cyclohexanols, prepared by the Tipson method,[5] were all shown to contain 94% or more of the theoretical amount of deuterium. Rates of acetolysis and pertinent data on the procedures and results are recorded in Table 1.

The most striking feature is the close similarity of the isotope effects with the *cis*-2-*d*- and *trans*-2-*d*-tosylates. Our isotope effects are qualitatively similar to those reported by Streitwieser,[3] though the numbers are somewhat larger. This fact would seem to indicate that there is little average difference in the dihedral angle between the C–OTs and C–D bonds for the *cis* and *trans* isomers in the cyclohexyl as well as in the cyclopentyl systems. The alternative, that the isotope effect shows little or no dependence on the dihedral angle, is rendered untenable by the work of Shiner and Jewett.[1,6]

This being the case, the conventional picture of solvolysis *via* a chair conformation requires that the solvolysis go essentially entirely through the conformation having the tosyloxy group equatorial,[7] which would predict similar dihedral angles for both isomers. Further, the isotope effects for axial–equatorial or equatorial–equatorial arrangements of the C–D and C–OTs bonds would have to be near 25%.

Both conditions are sufficiently unlikely that solvolysis of the unsubstituted cyclohexyl tosylate *via* a transition state having a twist-boat conformation becomes a much more attractive explanation. Shiner and Jewett[2] offer strong evidence for such a transition state in the solvolysis of *trans*-4-*t*-butylcyclohexyl brosylate.[2] In our more conformationally mobile system, the average dihedral angles in twist-boat conformations for the *cis*- and *trans*-2-*d* isomers should show similar depar-

(4) H. C. Brown and G. Zweifel, *ibid.*, 81, 247 (1959); 83, 2544 (1961).
(5) R. S. Tipson, *J. Org. Chem.*, 9, 235 (1944).
(6) V. J. Shiner, Jr., and J. G. Jewett, *J. Am. Chem. Soc.*, 86, 945 (1964).
(7) S. Winstein and N. J. Holness [*ibid.*, 77, 5562 (1955)], however, present data suggesting that *ca.* 70% of the solvolysis goes through the equatorial conformation, assuming a chair-like transition state.

tures from a parallel arrangement (*syn* for the *cis* and *anti* for the *trans*) and hence similar isotope effects.[8]

Acknowledgments. This work was supported by the National Science Foundation. We are indebted to Dr. V. J. Shiner, Jr., for informing us of his results prior to publication.

William H. Saunders, Jr., Kay T. Finley

Department of Chemistry, University of Rochester
Rochester, New York

Received December 12, 1964

3,4-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione. An Intermediate in the Microbiological Degradation of Ring A of Androst-4-ene-3,17-dione

Sir:

Previous studies have shown that one degradative pathway of androst-4-ene-3,17-dione (I) by microorganisms may be envisaged as follows[1,2]: androst-4-ene-3,17-dione (I) → 9α-hydroxyandrost-4-ene-3,17-dione (II) or androsta-1,4-diene-3,17-dione (III) → 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (IV) → 3aα-H-4α-[3'-propionic acid]-7$\alpha\beta$-methylhexahydro-1,5-indandione (V) → CO_2 + H_2O.

As large-scale fermentations have failed to yield intermediates which gave an insight as to the mechanism of conversion of IV into V, an alternate approach to this problem was undertaken. It is well documented that before a benzene ring is opened by means of bacterial enzymes which degrade aromatic compounds, two hydroxyl groups must first be introduced into the nucleus.[3,4] It has also been shown that, in some bacteria, the methyl group of *p*-cresol must be first oxidized to a carboxyl group before hydroxylation could take place on the aromatic ring.[5] Since the rate-determining step in the over-all breakdown of aromatic compounds by bacteria is usually the hydroxylation step, it would seem desirable to prepare 3,19-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VI), 2,3-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VII), and 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VIII). The availability of these compounds would then allow us to evaluate which one, if any, of these compounds is the likely intermediate in the fission of the aromatic ring.

When 6,19-oxidoandrost-4-ene-3,17-dione[6] was exposed to *Nocardia restrictus* (ATCC 14887), 9α-hydroxy-6,19-oxidoandrost-4-ene-3,17-dione was obtained, m.p. 260–266° dec., [α]^{26}D +79°, λ_{max}^{alc} 240 mμ (ϵ 11,900), λ_{max}^{Nujol} 2.86, 5.78, 6.02, and 6.18 μ. When the epoxide was treated with zinc dust, 9α,19-dihydroxyandrost-4-ene-3,17-dione was obtained, m.p. 256.5–258.5°, [α]^{29}D

+166°, λ_{max}^{alc} 245 mμ (ϵ 14,700), λ_{max}^{Nujol} 2,86, 2.95, 5.79, 6.08, and 6.21 μ. The latter compound was aromatized by reaction with *N. restrictus* in the presence of phenazine methosulfate to give VI, m.p. 140–141°, [α]^{25}D +89°, λ_{max}^{alc} 278 mμ (ϵ 2000), λ_{max}^{KBr} 2.98, 5.78, 5.84, 6.20, 6.31, and 6.66 μ. However, VI was only metabolized at a rate equal to IV by *N. restrictus* as measured by their rate of disappearance.

The simplest procedure that we could visualize for the preparation of VII would appear to involve the introduction of a 9α-hydroxyl group into 2-hydroxyandrosta-1,4-diene-3,17-dione; the resulting vinylog of a β-hydroxy ketone should then undergo reverse aldolization to yield VII.[7] However, when 2,17β-dihydroxyandrosta-1,4-dien-3-one[8] was exposed to *N. restrictus*, the major product obtained was 2ξ,9α-dihydroxyandrost-4-ene-3,17-dione, m.p. 240–243°, λ_{max}^{alc} 243 mμ (ϵ 12,000), [α]^{35}D +9°, λ_{max}^{Nujol} 2.90, 5.78, 6.05, and 6.20 μ. In an attempt to block the hydrogenase activity of this organism, 2-methoxy-17β-hydroxyandrosta-1,4-dien-3-one[9] (IX) was prepared with a view to obtaining 2-methoxy-3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (X) *via* an analogous 9α-hydroxylation procedure. When IX was exposed to *Nocardia corallina*[10] (ATCC 13259), surprisingly a phenolic compound with all the characteristics of 2-methoxy-3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (XI) was obtained, m.p. 185–188°, [α]^{25}D +135°, λ_{max}^{alc} 273 mμ (ϵ 900), λ_{max}^{KBr} 2.95, 5.78, 5.85, 6.15, 6.25, and 6.65 μ; n.m.r.[11] peaks τ 8.83 (3 H, one tertiary CH_3), 7.72 (3 H, one CH_3 on aromatic ring), 6.17 (3 H, one aromatic OCH_3), 4.62 and 3.58[12] (2 H, two phenolic OH), and 3.71 (1 H, one aromatic H). The catechol structure was further confirmed by its instability to base and a quinone was obtained after its oxidation by means of Ag_2O or mushroom tyrosinase. Thus, the synthesis of VII has lost its relevance to the problem since it appeared that VIII might be a more likely intermediate in the aromatic cleavage reaction.

Treatment of 9α,17β-dihydroxyandrost-4-en-3-one[13] (XII) with alkaline H_2O_2 resulted in the formation of 9α,17β-dihydroxy-4,5-oxidoandrostan-3-one (XIII), m.p. 197–198.5°, [α]^{26}D −66°, λ_{max}^{Nujol} 2.78, 2.86, and 5.85 μ. The epoxide was opened by its reaction with a sulfuricacetic acid mixture to yield 4,9α,17β-trihydroxyandrost-4-en-3-one (XIV), m.p. 227–229°, [α]^{29}D +42°, λ_{max}^{alc} 278 mμ (ϵ 12,700), λ_{max}^{Nujol} 2.87, 6.03, and 6.14 μ. When XIV was incubated with frozen cells of *N. restrictus* in the presence of phenazine methosulfate, VIII was obtained, m.p. 164–165°, [α]^{28}D +149°, λ_{max}^{alc} 282 mμ (ϵ 1950), $\lambda_{max}^{CHCl_3}$ 2.86, 3.00, 5.76, 5.92, 6.19, 6.26, and 6.69 μ.[14]

(1) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, 83, 4627 (1961).

(2) C. J. Sih and K. C. Wang, *ibid.*, 85, 2135 (1963).

(3) S. Dagley, P. J. Chapman, D. T. Gibson, and J. M. Wood, *Nature*, 202, 775 (1964).

(4) O. Hayaishi, M. Katagiri, and S. Rothberg, *J. Biol. Chem.*, 229, 905 (1957).

(5) S. Dagley and M. D. Patel, *Biochem. J.*, 66, 227 (1957).

(6) H. Ueberwasser, K. Heusler, J. Kalvoda, Ch. Meystre, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, 46, 344 (1963).

(7) C. J. Sih and A. M. Rahim, *J. Pharm. Sci.*, 52, 1075 (1963).

(8) P. N. Rao and L. R. Axelrod, *J. Am. Chem. Soc.*, 82, 2830 (1960).

(9) J. S. Baran, *ibid.*, 80, 1687 (1958).

(10) Among the 9-hydroxylating organisms tested (*N. restrictus, Pseudomonas testosteroni, Bacterium cyclooxydans, and Mycobacterium rhodochrous*), this *Nocardia corallina* gave the best yield of the product.

(11) All n.m.r. spectra were determined on a Varian Associates recording spectrometer (A-60) at 60 Mc. in deuterated chloroform. Chemical shifts are reported in τ-values (p.p.m.) [G.V.D. Tiers, *J. Phys. Chem.*, 62, 1151 (1958)].

(12) These two peaks disappeared after the addition of HCl.

(13) C. J. Sih, *J. Org. Chem.*, 26, 4716 (1961).

(14) Satisfactory analyses have been obtained on all the new compounds cited herein. We thank Mr. Joseph Alicino, Metuchen, N. J., for the analyses.