constancy of the entropies of activation for rate constants differing by about five orders of magnitude is suggestive (but certainly not conclusive) that a single mechanism, presumably the direct bimolecular attack of water at the central carbon atom, is involved.

The results of Figure 1 are the more remarkable in light of the relatively precise (equilibrium) linear free energy relationship which has been obtained for these *para*-substituted trityl cations between pK_{R^+} values and corresponding log K_{R-R} values for the reaction

$$R^+ + 0.5(R_0\text{-}R_0) \rightleftharpoons R_0^+ + 0.5(R\text{-}R)$$

The log K_{R-R} values have been obtained from emf cell measurements in acetonitrile solution.[6] Figure 2 illustrates this linear free energy relationship. Linear free energy relationships between rate and equilibria for analogous reactions (rather than for the same reaction as in Figure 1) have also been previously reported, but the ranges of cation structures have been limited.[7]

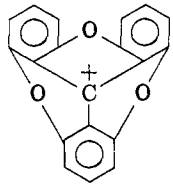
It is apparent from the dashed lines (slope 0.5) drawn in Figure 1, for example, that structurally limited linear free energy relationships may exist (the lines shown are not the only existing possibilities).[8] Further work is in progress to define the structural considerations relevant to rate *vs.* equilibrium in this reaction. However, the results in Figure 1 do suffice to support the general conclusion that, in the absence of collaborating evidence, arguments regarding cation stability based upon solvolysis rates (k_r corresponds to such a process) should be regarded as tenuous.

Acknowledgment. The authors wish to acknowledge the helpful assistance of Dr. N. Sutin of the Brookhaven National Laboratory in the use of his stop-flow reactor for conducting preliminary rate measurements.

(6) (a) E. D. Jenson and R. W. Taft, *J. Am. Chem. Soc.*, **86**, 116 (1964); (b) R. W. Taft and L. D. McKeever, *ibid.*, **87**, 2489 (1965); (c) Ph.D. Thesis of L.D. McKeever, University of California, Irvine, Sept 1966.

(7) (a) N. C. Deno, G. Sains, and M. Spangler, *J. Am. Chem. Soc.*, **84**, 3295 (1962); (b) N. Lichten, *Progr. Phys. Org. Chem.*, **1**, 75 (1963); (c) R. Ginter and S. F. Mason, *Trans. Faraday Soc.*, **60**, 882 889 (1964); (d) H. Dauben Jr., private communication.

(8) As drawn in Figure 1, the cations with only oxygen substituents react about *two powers* of ten faster than nitrogen-substituted cations of equal pK_{R^+}. We have also noted that the Martin cation



(J. C. Martin and R. G. Smith, *J. Am. Chem. Soc.*, **84**, 2252 (1964)), of very similar pK_{R^+} (+9.05) to that of crystal violet, reacts with water 4.3 log units faster.

(9) Taken in part from the Ph.D. Thesis of R. A. Diffenbach, The Pennsylvania State University, Sept 1966.

(10) Grateful acknowledgment is made to the Fuji Photo Film Co., Ltd., Kanagawa, Japan, for support of K. S. during the period of this research at The Pennsylvania State University.

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The Mechanism of the Chromic Acid Oxidation of Ketones. Kinetic Proof for an Enol Intermediate

Sir:

Several groups of investigators have suggested that the chromic acid oxidation of a ketone proceeds *via* an enol intermediate.[1-3] However, until now no conclusive experimental evidence for this view has been offered. Mechanism 1

$$\text{ketone} \underset{k_K}{\overset{k_E}{\rightleftharpoons}} \text{enol} \xrightarrow[k]{CrO_3} \text{product} \qquad (1)$$

leads to the rate expression 2

$$\frac{-d[CrO_3]}{dt} = \frac{k_E k[\text{ketone}][CrO_3]}{k_K + k[CrO_3]} \qquad (2)$$

where k_E, k_K, and k are the rate constants for enolization, ketonization, and oxidation, and [ketone] and [CrO_3] denote the total analytical concentrations of the ketone and of hexavalent chromium, respectively.

Rate eq 2 predicts a change from rate-limiting oxidation to rate-limiting enolization when $k[CrO_3]$ becomes much larger than k_K and, consequently, a change from first- to zero-order dependence in hexavalent chromium. Furthermore, the limiting oxidation rate should be of the same order and very approximately of the same magnitude as the rate of enolization.[4] However, none of the previously investigated chromic acid oxidations of ketones exhibited zero-order kinetics. In the case where enolization and oxidation rates were compared,[3] the enolization was found to be considerably faster.

We found now two ketones, isobutyrophenone and 2-chlorocyclohexanone, in which the relative rate of oxidation to enolization is favorable enough to allow the observation of the predicted change in the rate-limiting step. Figure 1 shows the dependence of the initial oxidations rates[5] (corrected for unit ketone concentration) as a function of the analytical concentration of chromic acid. Both curves demonstrate clearly the change from first- to zero-order dependence as required by the above mechanism. Furthermore, the oxidation rates reach a value of about 0.67 of the enolization rate for isobutyrophenone[6] and of approximately 2.2 for 2-chlorocyclohexanone. Both of these findings are fully in accord with the kinetic consequences of eq 2

(1) E. Wenkert and B. G. Jackson, *J. Am. Chem. Soc.*, **80**, 211 (1958).

(2) K. Umeda and K. Tarama, *Nippon Kagaku Zasshi*, **83**, 1216 (1962).

(3) P. A. Best, J. S. Littler, and W. A. Waters, *J. Chem. Soc.*, 822 (1962).

(4) The reason why only a similarity and not an exact identity of enolization and oxidation rates can be expected lies in the different units used to measure the rate of the two reactions. While enolization rates are expressed in changes of the concentration of the ketone in a unit of time, oxidation rates use out of necessity changes in the concentration of the oxidant. The two scales can be interconverted only if the exact stoichiometry of the oxidation reaction is known. The oxidation rate will be two-thirds of the enolization rate if only one oxygen atom is consumed for each molecule of ketone which has reacted; it will be 1.3, 2.0, 2.7, etc., times higher than the rate of enolization if an average of two, three, four, etc., oxygen atoms is used to oxidize the ketone to stable oxidation products.

(5) The rates were measured spectrophotometrically in a thermostated cell holder of a Carl Zeiss PMQ II spectrophotomer at 350 mμ.

(6) Enolization rates were determined from bromination studies under the assumption that only the monobromo derivative is formed in the presence of excess ketone. Bromination rates were measured spectrophotometrically; very good zero-order plots were generally obtained.

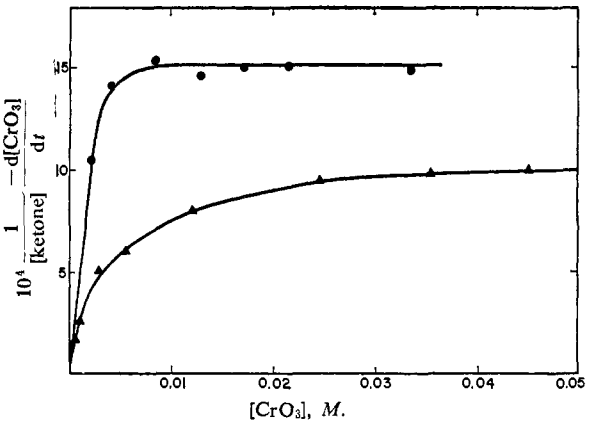

Figure 1. Effect of the initial concentration of chromic acid on the rate of oxidation at 30°. Circles, isobutyrophenone in 0.50 M $HClO_4$ in 99% acetic acid; triangles, 2-chlorocyclohexanone in 1.0 M aqueous $HClO_4$.

and therefore very strongly support the enolization mechanism.

As these results were obtained from two structurally very different ketones in two different solvent media and as no monoketone is known to undergo oxidation which is considerably faster than its enolization,[7] we believe these results support the assumption that enolization generally precedes oxidation in the reaction of chromic acid with a monoketone. In this respect then chromic acid reacts similarly as thalic, mercuric, and permanganate salts[8] and also as manganic pyrophosphate,[9] but differs from cobaltic[10,11] and ceric[11,12] salts and from manganic sulfate[11] as oxidants.

Acknowledgment. Support of this research through No. 1524-A4, a grant of the Petroleum Research Fund of the American Chemical Society, is gratefully acknowledged.

(7) Sr. A. Riehl, Thesis, The Catholic University of America, Washington, D. C., 1965.
(8) J. S. Littler, *J. Chem. Soc.*, 827 (1962).
(9) A. Y. Drummond and W. A. Waters, *ibid.*, 497 (1955).
(10) D. G. Hoare and W. A. Waters, *ibid.*, 971 (1962).
(11) J. S. Littler, *ibid.*, 832 (1962).
(12) S. Venkatakrishnan and M. Santhappa, *Z. Physik. Chem.* (Frankfurt), **16**, 73 (1958).
(13) Address correspondence to Department of Chemistry, University of Illinois at Chicago Circle, Chicago, Ill. 60680.

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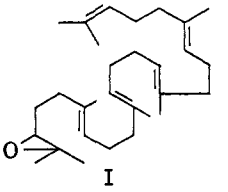
2,3-Oxidosqualene, an Intermediate in the Biological Synthesis of Sterols from Squalene[1]

Sir:

There is now ample evidence for the role of squalene as a precursor of sterols and polycyclic triterpenes and for the relationships between the carbon skeleton of squalene and the structures of many of the polycyclic natural products derived therefrom.[2] However, much remains to be learned about the details of these remarkable cyclization processes. This note is concerned with the question of how cyclization of squalene is initiated and with the connection between this cyclization process and the origin of the characteristic 3-hydroxyl group of sterols and triterpenes. There are three general types of mechanisms which can reasonably account for the cyclization of squalene and the introduction of the 3-hydroxyl substituent: (1) attack at C_3 by a reagent which transfers OH^+ with concurrent initiation of cyclization from C_2;[3] (2) proton transfer to C_3 to initiate cyclization to a 3-deoxycyclosqualene followed by subsequent hydroxylation at the saturated 3-methylene group of the cyclization product;[4] and (3) introduction of oxygen at C_3 to give an intermediate which is capable of cyclization in a separate step. Evidence has been obtained which strongly supports the last of these propositions and indicates specifically



I

that the 2,3-oxidosqualene (I) is an intermediate in the biosynthesis of sterols from squalene.

The biosynthetic experiments were carried out using rat liver homogenates[5] and a number of radioactive substrates, including [^{14}C]squalene[6] and racemic [^{14}C]2,3-oxidosqualene (I).[7]

Using rat liver homogenate under standardized aerobic conditions which convert squalene to sterols, racemic [^{14}C]2,3-oxidosqualene gave rise to a radioactive sterol fraction which was isolated by preparative layer chromatography (R_f corresponding to cholesterol) and shown by vapor phase chromatography (vpc) and comparison with authentic samples to consist of cholesterol and lathosterol (cholest-7-en-3β-ol) in a ratio of *ca.* 1:2.[8] Under these aerobic conditions, which gave 62% conversion of [^{14}C]squalene to sterols, 40% of the racemic [^{14}C]2,3-oxidosqualene was transformed into labeled sterols (80% conversion based on a *single* antipode). The conversion of [^{14}C]2,3-oxidosqualene to sterols was essentially unchanged when the standard incubation was carried out *anaerobically* under argon whereas the incorporation of [^{14}C]squalene into sterols dropped to only 13% in the anaerobic system.[9] The

(1) In this note the names used for squalene derivatives have been obtained by assigning the numbers 1–24 to the carbons along the chain from one terminal methyl group to the other. Thus, 2,3-oxidosqualene corresponds to 2,3-oxido-2,6,10,15,19,23-hexamethyl-6,10,14,18,22-tetracosapentaene.
(2) For a recent review, see R. B. Clayton, *Quart. Rev.* (London), **19**, 168 (1965).
(3) See L. Ruzicka, A. Eschenmoser, and H. Heusser, *Experientia*, **9**, 357 (1953); A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).
(4) Arguments in favor of this possibility have been given recently by D. H. R. Barton and G. P. Moss, *Chem. Commun.*, 261 (1966).
(5) Prepared by the method of N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.*, **222**, 1 (1956).
(6) Prepared by anaerobic incubation of rat liver homogenate[5] with DL-[2-^{14}C]mevalonic acid dibenzylethylenediamine salt and purified chromatographically.
(7) [^{14}C]Squalene[6] was converted to the 2,3-bromohydrin using the method of E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962), which employs hypobromous acid in aqueous glyme. The monobromohydrin was purified by preparative layer chromatography (plc) on silica gel and transformed by treatment with ethanolic base to racemic [^{14}C]2,3-oxidosqualene, homogeneous on thin layer chromatography. An analysis of the oxide by high-resolution mass spectrometry indicated a molecular weight of 426.3860 (calcd for $C_{30}H_{50}O$: 426.3861).
(8) Vpc analyses were conducted on an F & M Model 400 biomedical instrument using a glass column (3 mm i.d.) 4 ft in length packed with 2% Epon 1001 on Diatoport S at 235°.