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Leslie F. Warren, Jr.,[11] M. Frederick Hawthorne
Department of Chemistry, The University of California
Riverside, California
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An Investigation of the Intermediates in the Base-Catalyzed Decomposition of Camphor Tosylhydrazone in Aprotic Solvents

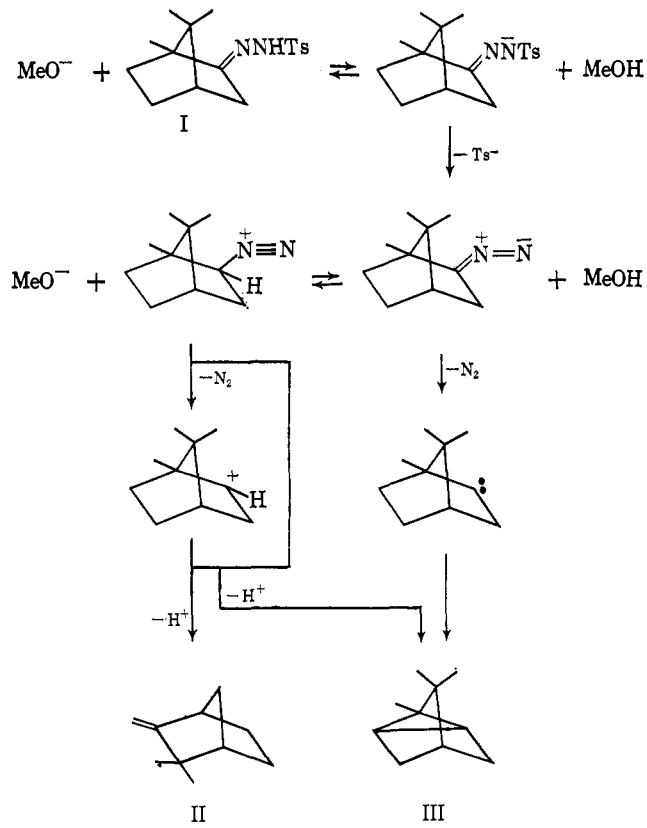
Sir:

We have recently investigated the effect of trivalent metal cations on the camphene:tricyclene ratio resulting from the base-catalyzed decomposition of camphor tosylhydrazone (I) in aprotic solvents.[1] The results of this investigation led us to conclude that the trivalent metal cation participates in the formation of an intermediate which behaves like a 2-bornyl cation and rearranges to camphene (II). The formation of tricyclene (III) was assumed to arise from a carbene intermediate *via* a transannular carbon–hydrogen insertion reaction, as has been reported in many investigations.[2]

We have been studying the effect of base concentration on the decomposition of camphor tosylhydrazone (I) with sodium methoxide and sodium hydride in aprotic solvents[3] and have obtained strong evidence that the nature of the intermediate which leads to tricyclene (III) is dependent upon the relative amount of base present in the reaction mixture.

In the base-dependence study[3] we discovered that the relative amount of camphene (II) to tricyclene (III) decreased at higher base concentrations. Friedman, *et al.*, made a similar observation in the decomposition of cyclopropanecarboxaldehyde tosylhydrazone with sodium methoxide.[4] When less than 1 equiv of base was used or when the reaction was run in ethylene glycol, bicyclobutane, presumably arising from a carbonium ion intermediate, was the major product.[4] Wiberg and Lavanish studied the decomposition of cyclopropanecarboxaldehyde tosylhydrazone in protic solvents and also concluded that the bicyclobutane was generated from a cationic intermediate.[5]

We allowed camphor tosylhydrazone (I) to decompose with 0.75, 2.00, and 4.00 equiv of sodium methoxide in diglyme in the presence of deuterium oxide (40 equiv). The camphene (II) and tricyclene (III) formed in the reaction were separated and collected by gas chromatography. The collected samples were split; half of the material was reinjected into the gas chromatograph as a check for purity, and the other half was submitted for mass spectral analysis. The camphene (II) from each reaction mixture contained 80 ± 2% d_1, the remainder being unlabeled. The tricyclene (III) from the 0.75-equiv reaction contained 64% d_1, that from the



2.00-equiv reaction 25% d_1, and that from the 4.00-equiv reaction 8% d_1. The first result is consistent with that obtained by Wiberg, who obtained labeled bicyclobutane from the decomposition of cyclopropanecarboxaldehyde tosylhydrazone with less than 1 equiv of base in ethylene glycol-d_2.[6]

Tricyclene (III) formation by way of a carbene does not lead to the incorporation of a deuterium atom, and therefore it is clear that with 0.75 equiv of sodium methoxide about two-thirds of the III is generated from some other intermediate, whereas at higher concentrations (*e.g.*, 4.00 equiv) the carbene mechanism appears to predominate. A cationic mechanism is consistent with deuterium incorporation at low base concentrations, and in fact the cationic formation of tricyclene is in complete accord with the report that bicyclobutane results from a cationic intermediate in the base-catalyzed decomposition of cyclopropanecarboxaldehyde tosylhydrazone.[4,5] These results are also consistent with other reports of cyclopropane formation from cationic intermediates,[7] especially those describing the formation of nortricyclene from the norbornyl cation[8] and norbornanediazonium ion[9] in protic solvents. However, in the base-catalyzed decomposition of camphor tosylhydrazone (I) the generated cyclopropane, III, can be formed from one of two intermediates depending on the concentration of base.

Considering first the decomposition of I with 0.75 equiv of base, the following explanation is consistent

(1) R. H. Shapiro, *Tetrahedron Letters*, 3401 (1966).
(2) See W. Kirmse, "Carbene Chemistry," Academic Press Inc., New N. Y., 1964, Chapter 3, for a review.
(3) R. H. Shapiro, J. H. Duncan, and J. C. Clopton, *J. Am. Chem. Soc.*, in press.
(4) J. A. Smith, H. Shechter, J. Bayless, and L. Friedman, *ibid.*, 87, 659 (1965).
(5) K. B. Wiberg and J. M. Lavanish, *ibid.*, 88, 365 (1966).
(6) Personal communication from Professor Wiberg, Aug 11, 1966. See K. B. Wiberg and J. M. Lavanish, *ibid.*, 88, 5272 (1966); F. Cook, L. Friedman, R. L. Foltz, and R. Randall, *ibid.*, 88, 3870 (1966).
(7) W. Hückel and G. Meinhardt, *Ber.*, 90, 2025 (1957); G. M. Komppa and G. A. Nyman, *Ann.*, 535, 252 (1938); M. Bredt-Savelsberg, *Ber.*, 56, 554 (1923); G. Wagner, S. Moycho, and F. Zienkowski, *ibid.*, 37, 1032 (1904).
(8) S. Winstein, E. Clippinger, R. Howe, and E. Vogelfanger, *J. Am. Chem. Soc.*, 87, 376 (1965).
(9) A. Nickon and N. H. Werstiuk, *ibid.*, 88, 4543 (1966).

with the experimental data. The tosylhydrazone I reacts with base reversibly to give the conjugate base of I and methanol. The anion loses *p*-toluenesulfinate, giving diazocamphane in the rate-determining step.[10,11] In the absence of excess strong base the intermediate diazocamphane attacks a proton either on the liberated methanol or on the tosylhydrazone which is present in abundance. The methoxide ion and tosylhydrazone again set up an equilibrium while the resulting diazonium decomposes directly to products,[9] or in a two-step process loses nitrogen and becomes a poorly solvated carbonium ion.[12] This carbonium ion can then collapse by two routes: (1) by a transannular 1,3-proton elimination to give tricyclene in low yield (*ca.* 35%), and (2) by a Wagner–Meerwein rearrangement and proton expulsion to give camphene in higher yield (*ca.* 65%).[3]

At higher base concentrations (*e.g.*, 4.00 equiv) the equilibrium between the tosylhydrazone and its conjugate base highly favors the latter, which decomposes to diazocamphane as previously mentioned. The diazocamphane can then abstract a proton from methanol to set up an equilibrium between methoxide ion and the resulting diazonium ion, the former being present in large excess. Therefore, in the presence of a large excess of methoxide the diazocamphane should be favored in the equilibrium, and this can then lose nitrogen, giving the carbene intermediate.

The reaction leading to camphene has its interesting aspects also. First the deuterium incorporation in the labeling experiments amounted to about 80% instead of the calculated 97.5% (99.7% D_2O in a 40-fold excess). This can be the result of two phenomena: (1) some of the camphene results from a carbene intermediate, or (2) the selectivity of the diazocamphane for hydrogen is about seven times that for deuterium. Because the amount of deuterium incorporation is independent of base concentration, the second explanation seems more reasonable and is being investigated further. Another interesting point is that the poorly solvated cation generated in our system, aside from giving tricyclene, undergoes rearrangement. This behavior is not consistent with that previously reported[12] where rearrangement of poorly solvated cations is apparently inhibited.

The results of this investigation have shown that "carbenic" products do not always result from carbene intermediates.[13] We are presently studying reactions such as the base-catalyzed dehydrohalogenation of isobornyl chloride in aprotic solvents in order to determine the generality of this phenomenon.

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(10) L. Friedman and H. Shechter, *J. Am. Chem. Soc.*, **81**, 5512 (1959).
(11) J. W. Powell and M. C. Whiting, *Tetrahedron*, 7, 305 (1959).
(12) A. T. Jurewicz, J. H. Bayless, and L. Friedman, *J. Am. Chem. Soc.*, **87**, 5788 (1965); J. H. Bayless, F. D. Mendicino, and L. Friedman, *ibid.*, **87**, 5790 (1965).
(13) See also J. W. Wilt, C. A. Schneider, H. F. Dabek, J. F. Kraemer, and W. J. Wagner, *J. Org. Chem.*, **31**, 1543 (1966), for a similar conclusion using N-methylpyrrolidone as a solvent.

Robert H. Shapiro, J. H. Duncan, J. C. Clopton
Department of Chemistry, University of Colorado
Boulder, Colorado 80304
Received August 24, 1966

A Method for Obtaining Three-Dimensional Structural Information about Protein Molecules in Solution

Sir:

Despite the noteworthy success of X-ray crystallography in obtaining complete three-dimensional structures for several protein molecules,[1] the possibility remains that protein conformations in aqueous solution under physiological conditions differ from those in the crystal. The observation of what are felt to be conformational changes in protein molecules in solution produced by changes in temperature and pH, or by allosteric effects induced by small molecules, indicates a degree of flexibility which might easily result in structural change as a result of packing into the crystalline lattice. The structural questions raised by these changes in solution are, of course, of great interest in themselves. We wish to report results leading toward a structural method applicable to proteins in aqueous solution under a variety of conditions.

Chloroform (85 mg) made from T_2O by the haloform reaction and containing 1 curie of tritium was distilled along with 2 ml of water into 200 mg of chymotrypsinogen A in the absence of air. The solution was then exposed to a ^{60}Co γ-ray source of 0.125 Mrad/hr for 30 min. After freeze drying and dialysis to remove exchangeable OH and NH protons, this material had an activity of 1,125,000 cpm/mg. A control run where the γ irradiation was omitted was then conducted. After freeze drying and dialysis 6000 cpm/mg was found. The tritiated chloroform was subsequently used successfully for several more labeling experiments.

The sample of chymotrypsinogen which had been labeled in the ^{60}Co source was chromatographed and rechromatographed on Amberlite GC-50 resin. Following the chromatography by ultraviolet spectroscopy, peaks of the same shape as those found using nonlabeled chymotrypsinogen were obtained. The radioactivity was found to follow the peak detected by ultraviolet spectroscopy. The activity of the rechromatographed labeled chymotrypsinogen was 611,000 cpm/mg. The decrease in specific activity is probably due to further exchange during dialysis of hydrogens such as those α to the amide carbonyls. Some radioactivity might have been present in chemically altered protein and been removed by chromatography, but this is less likely. Repetition of the chromatography on Sephadex G-50 gave similar results.

We feel that these results indicate that a considerable amount of labeling through exchange of C–H hydrogens for tritium has occurred, presumably through abstraction of hydrogen atoms from the protein by the OH radicals produced by irradiation of water, followed by donation of tritium atoms to the protein radicals by the chloroform. The chromatographic evidence indicates that it is likely that the exchange was accomplished without structural damage to the protein.

Because of the size of the $CTCl_3$ molecule, we feel that this labeling process could occur readily only on the *outside* of the protein molecule. Larger donors of tritium might be used, if necessary, to make a better distinction between inside and outside. With labeling

(1) J. C. Kendrew, *Science*, **139**, 1259 (1963); M. Perutz, *ibid.*, **140**, 863 (1963); D. C. Phillips, *Sci. Am.*, **215**, No. 5, 78 (1966).