BIOORGANIC CHEMISTRY **25,** 239–245 (1997)
ARTICLE NO. BH971069

Mechanistic Imperatives for the Reaction Catalyzed by Isopentenyl Pyrophosphate Isomerase: Free Energy Profile for Stepwise Isomerization in Water through a Tertiary Carbocation Intermediate[1]

Maria M. Toteva and John P. Richard[2]

Department of Chemistry, University at Buffalo, SUNY, Buffalo, New York 14260-3000

Received May 23, 1997

Rate and equilibrium constants are reported for the stepwise allylic 1,3-isomerization of 4-(4-methoxyphenyl)-2-methyl-1-butene (**2**) to give 4-(4-methoxyphenyl)-2-methyl-2-butene (**3**) in water through a simple tertiary carbocation intermediate $\mathbf{1}^+$, and the data are used to construct a free energy profile for the reaction. This profile shows that isopentenyl pyrophosphate isomerase stabilizes the carbocation-like transition state for the stepwise isomerization of simple alkenes by ca. 16 kcal/mol. The barriers for the deprotonation of the tertiary carbocation $\mathbf{1}^+$ by solvent water are significantly smaller than those for the protonation of simple enolates by this solvent. This difference favors a concerted mechanism for the enzyme-catalyzed 1,3-isomerization of alkenes, which avoids the formation of a tertiary carbocation intermediate.

INTRODUCTION

The mechanisms by which enzymes stabilize putative bound enol(ate)s is a subject of considerable recent interest (*1–5*). However, much less attention has been focused on understanding the mechanism for the stabilization of carbocation-like transition states of enzymatic reactions, and there have been no comparisons of the energetics of a reaction which proceeds through an aliphatic carbocation intermediate in water with those for the corresponding enzyme-catalyzed process. We report here the free energy reaction coordinate profile for the nonenzymatic allylic 1,3-isomerization of the simple alkene **2** to give **3** at pH 7.0, which proceeds through the simple tertiary carbocation $\mathbf{1}^+$ (Scheme 1). This profile allows an estimate of the rate acceleration for the reaction catalyzed by isopentenyl pyrophosphate isomerase, and it defines the mechanistic imperatives for enzymatic catalysis of the 1,3-isomerization of simple alkenes.

EXPERIMENTAL SECTION

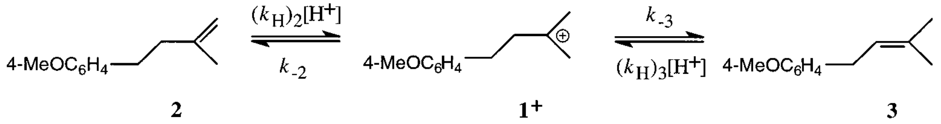
The materials and procedures for the synthesis of **2,** along with spectral data for this compound, are given in an earlier publication (*6*).

---

[1] This work was supported by a grant from the National Institutes of Health (GM 39754).

[2] To whom correspondence should be addressed.

0045-2068/97 $25.00



SCHEME 1

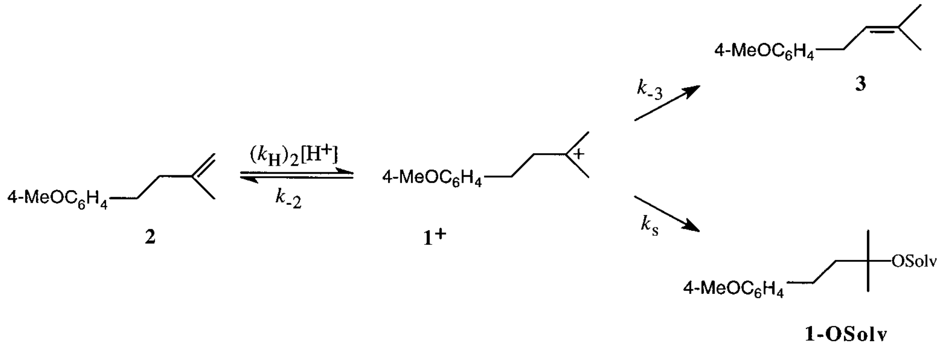
Product and kinetic studies. These reactions were carried out in 20 : 80 (v/v) TFE/H_2O (TFE, trifluoroethanol) at 37°C and a constant ionic strength of 0.80 maintained with sodium perchlorate. The reactions were initiated by making a 100-fold dilution of a solution of **2** in acetonitrile into the reaction mixture to give a final substrate concentration of ca. 7×10^{-4} M. The products of acid-catalyzed reactions of **2** were identified and their yields were quantified by HPLC analysis as described in earlier work (*6*). The rate constant ratios for partitioning of the reaction intermediate $\mathbf{1}^+$ between loss of a proton and addition of solvent was determined according to Eq. [1], where [**3**]/([**1-OSolv**]) is the ratio of the yields of the products of 1,3-isomerization and solvolysis reactions of **2.**

$$k_{-3}/k_s = [\mathbf{3}]/([\mathbf{1\text{-}OSolv}]) \qquad [1]$$

The reaction of **2** in 20 : 80 (v/v) TFE/H_2O containing perchloric acid was followed by HPLC by monitoring the decrease in the area of the peak for **2** using 9-methoxyfluorene (4×10^{-5} M) as an internal standard. The first-order rate constants for the disappearance of **2** were determined as the slope of a semilogarithmic plot of reaction progress against time which covered three reaction halftimes.

RESULTS

The second-order rate constant for the acid-catalyzed reaction of **2** to form **3** and adducts to the water and trifluororethanol cosolvents (**1-OSolv,** Scheme 2) in 20 : 80 (v/v) TFE/H_2O at 37°C and I = 0.80 ($NaClO_4$), determined as the slope of



SCHEME 2

a plot of k_{obsd} against [$HClO_4$], is $(k_H)_{obsd} = 6.5 \times 10^{-4}$ M^{-1} s^{-1}. The alkene **2** was not sufficiently soluble to allow for determination of this second-order rate constant in water, the solvent used in studies of the isomerization reaction catalyzed by isopentenyl pyrophosphate isomerase. However, the change from 50:50 (v/v) to 20:80 (v/v) TFE/H_2O results in only a small, 20%, increase in the value of $(k_H)_2$ at 25°C, from 8.1×10^{-5} (*6*) to 9.8×10^{-5} M^{-1} s^{-1} so that this rate constant varies only slightly as trifluoroethanol in the solvent is replaced by water.

The yield of the alkene **3** from the acid-catalyzed reactions of **2** in 20:80 (v/v) TFE/H_2O is 3%. A product rate constant ratio of $k_{-3}/k_s = 0.031$ for partitioning of $\mathbf{1}^+$ between loss of a proton to form **3** and nucleophilic addition of water to form **1-OSolv** in 20:80 (v/v) TFE/H_2O (Scheme 2) was determined from the ratio of the yields of the products **3** and **1-OSolv** from the acid-catalyzed reaction of **2** (Eq. [1]). A product rate constant ratio of $k_{-2}/k_s = 0.013$ for partitioning of $\mathbf{1}^+$ between loss of a proton to form **2** and nucleophilic addition of solvent in 20:80 (v/v) TFE/H_2O (Scheme 2) was estimated from $k_{-3}/k_s = 0.031$ and the product rate constant ratio of $k_{-3}/k_{-2} = 2.4$ determined as the ratio of the alkenes **3** and **2** obtained as products of the acid-catalyzed reaction of **1-OH** in 20:80 (v/v) TFE/H_2O at 25°C (*6*).

DISCUSSION

The rate constants for the reversible isomerization of **2** to **3** through a tertiary carbocation reaction intermediate (Scheme 1) have been directly determined, or estimated, from the rate and product data as follows.

(1) A value of $(k_H)_2 = 6.6 \times 10^{-4}$ M^{-1} s^{-1} (Table 1) for protonation of **2** by solvent to form $\mathbf{1}^+$ was estimated using the relationship $(k_H)_2 = (k_H)_{obsd}/(1 - f_2)$, where $f_2 = 0.012$ is the fraction of $\mathbf{1}^+$ that partitions to reform **2** (Scheme 2). This value of $f_2 = 0.012$ was calculated from the observed 3% yield of **3** from the reaction of **2** and the product rate constant ratio of $k_{-3}/k_{-2} = 2.4$ which is the ratio of the alkenes **3** and **2** from the acid-catalyzed reaction of **1-OH** in 20:80 (v/v) TFE/H_2O at 25°C (*6*). We assume that the rate constant ratio will be the same for reactions at 25 and 37°C because the increase in temperature from 25 to 37°C does not affect the partitioning rate constant ratios for the reaction of **2** in 20:80 (v/v) TFE/H_2O.

(2) In mostly aqueous solutions the tertiary carbocation $\mathbf{1}^+$ partitions between very fast nucleophilic addition of solvent to form **1-OSolv,** k_s (s^{-1}), and slower deprotonation by solvent to form **2** and **3** (*6*). There is good evidence that nucleophilic addition of solvent to $\mathbf{1}^+$ is so fast that the rate constant k_s is limited by the rate of rotation of a solvent molecule into a reactive conformation, so that $k_s = k_{reorg} \approx 10^{11}$ s^{-1} (*7*), where k_{reorg} is the rate constant for reorganization of the local solvation shell of the carbocation by the dielectric relaxation of solvent. The value of $k_s = 10^{11}$ s^{-1} was combined with the product rate constant ratios $k_{-2}/k_s = 0.013$ and $k_{-3}/k_s = 0.031$ determined for the partitioning of $\mathbf{1}^+$ between loss of a proton and the addition of solvent, to give $k_{-2} = 1.3 \times 10^9$ s^{-1} and $k_{-3} = 3.1 \times 10^9$ s^{-1} for the deprotonation of $\mathbf{1}^+$ by solvent to form the alkenes **2** and **3,** respectively (Table 1).

(3) The value of $(k_H)_3 = 1.6 \times 10^{-4}$ M^{-1} s^{-1} was determined from the value of