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A thermodynamic study of the ketoreductase-catalyzed reduction of 2-alkanones in non-aqueous solvents

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Abstract

Equilibrium constants K have been measured for the reactions (2-alkanone + 2-propanol = 2-alkanol + acetone), where 2-alkanone = 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-octanone and 2-alkanol = 2-butanol, 2-pentanol, 2-hexanol, 2-heptanol, and 2-octanol. The solvents used were *n*-hexane, toluene, methyl *tert*-butyl ether (MTBE), and supercritical carbon dioxide $SCCO_2$ (pressure $P = 10.0$ MPa). The temperature range was $T = (288.15$ to $308.27)$ K. Chiral analysis of the reaction products showed that the enzyme used in this study was stereoselective for the 2-octanone reaction system, *i.e.* only (*S*)-(+)-2-octanol was formed. For the reactions involving butanone, pentanone, and hexanone, the products were racemic mixtures of the respective (*S*)-(+)-2-alkanol and the (*R*)-(−)-2-alkanol. Chiral analysis showed that for the 2-heptanone reaction system, the 2-alkanol product was a mixture of (*S*)-(+)-2-heptanol and (*R*)-(−)-2-heptanol, at the respective mole fractions of 0.95 and 0.05. The equilibrium constant for the reaction system involving 2-butanone carried out in *n*-hexane was measured at several temperatures. For this reaction, the values for the thermodynamic reaction quantities at $T = 298.15$ K are: $K = 0.838 \pm 0.013$; the standard molar Gibbs free energy change $\Delta_r G^\circ_m = (0.44 \pm 0.040)$ kJ · mol^{-1}; the standard molar enthalpy change $\Delta_r H^\circ_m = -(1.2 \pm 1.7)$ kJ · mol^{-1}, and the standard molar entropy change $\Delta_r S^\circ_m = -(5.5 \pm 5.7)$ J · K^{-1} · mol^{-1}. Interestingly, inspection of the values of the equilibrium constants for these reactions carried out in *n*-hexane, toluene, MTBE, and $SCCO_2$ shows that these values are comparable and have little dependence on the solvent used to carry out the reaction. The values of the equilibrium constants decrease monotonically with increasing value of the number of carbons N_C and trend towards a limiting value of ≈0.30 for $N_C > 8$.
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1. Introduction

Enzyme-catalyzed reactions in organic solvents have proven to be useful for the stereoselective synthesis of chiral intermediates for pharmaceuticals and agrochemicals [1–3]. Lipase-catalyzed esterification [4–6] and transesterification [1,7] reactions in organic solvents have been commonly used for the enantioselective resolution of racemic mixtures. Recently, ketoreductase-catalyzed reactions [8–12] have been used for the production of chiral secondary alcohols from the corresponding ketones. These chiral alcohols are useful intermediates for the pharmaceutical, agrochemical, and perfume industries. For several years, our laboratory has been interested in the thermodynamics [13–18] of enzyme-catalyzed reactions in organic solvents. Recently, our studies have been extended to include enzyme-catalyzed reactions carried out in supercritical carbon dioxide ($SCCO_2$) [19]. Supercritical carbon dioxide is an attractive solvent for biocatalysis [20–22] because of

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its easy removal from the reaction mixture, non-toxicity, non-flammability, abundant availability, environmental friendliness, and recyclability. The low viscosity and high diffusivity of $SCCO_2$ also help to provide favorable mass transfer properties, which can enhance the catalysis. The thermodynamic results obtained in these studies are essential for the basic understanding of the energetics of these reactions and also for the practical utilization of these enzyme-catalyzed reactions in these solvents.

In the present investigation, we report results of equilibrium measurements for the following ketoreductase-catalyzed transhydroxylation reactions (see figure 1)

2-butanone(soln) + 2-propanol(soln) = 2-butanol(soln) + acetone(soln), (1)

2-pentanone(soln) + 2-propanol(soln) = 2-pentanol(soln) + acetone(soln), (2)

2-hexanone(soln) + 2-propanol(soln) = 2-hexanol(soln) + acetone(soln), (3)

2-heptanone(soln) + 2-propanol(soln) = 2-heptanol(soln) + acetone(soln), (4)

2-octanone(soln) + 2-propanol(soln) = 2-octanol(soln) + acetone(soln). (5)

Here "soln" denotes any of the four organic solvents used in this study.

The activity of the ketoreductase depends on the presence of a small catalytic amount of β-nicotinamide-adenine dinucleotide (reduced) {NADP(red)}. The ketoreductase-catalyzed reaction proceeds in two steps. In the first step, the 2-alkanone is reduced to the corresponding 2-alkanol and NADP(ox). In the second step, the NADP(ox) is reduced to NADP(red):

2NADP(red) + 2-alkanone = 2-alkanol + 2NADP(ox), (6)

2NADP(ox) + 2-propanol = 2NADP(red) + acetone. (7)

Combination of equations (6) and (7) gives the overall reaction for the reduction of the 2-alkanone

2-alkanone + 2-propanol = 2-alkanol + acetone. (8)

The principal aims of this study were to determine how the values of the equilibrium constants for the above reactions vary as a function of the number of carbons in the respective alkanones and also to examine

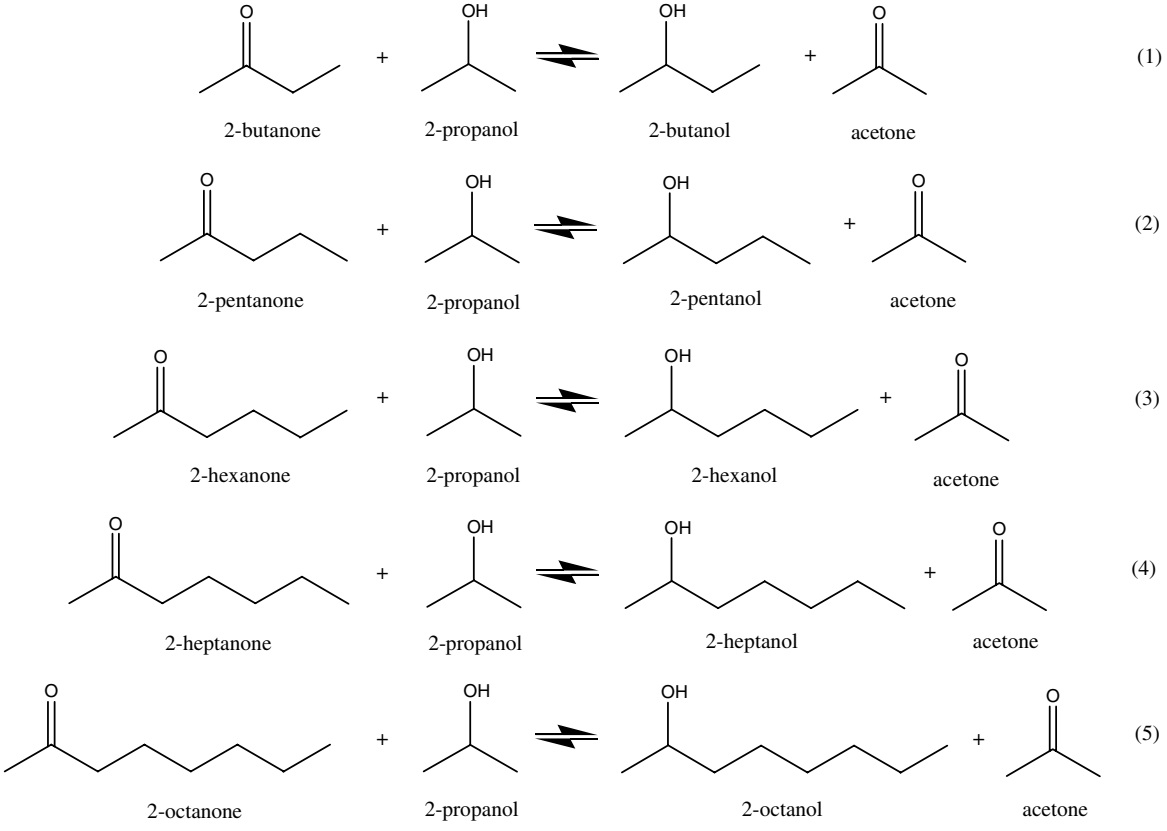

FIGURE 1. Structures of the substances in reactions (1) to (5).

how the values of these equilibrium constants depend on the solvent. The equilibrium constants for reaction (1) were measured by using *n*-hexane, toluene, methyl *tert*-butyl ether (MTBE), and supercritical carbon dioxide ($SCCO_2$) as the solvents. The temperature dependency of the equilibrium constant for reaction (1) in *n*-hexane was also measured. Reaction (2) was studied by using hexane and $SCCO_2$ as solvents. Reactions (3) and (4) were studied by using *n*-hexane as the solvent. Finally, reaction (5) was studied by using *n*-hexane, toluene, and MTBE as the solvents. These solvents were selected based upon their current and potential use in non-aqueous enzymology [3].

In all cases, the chirality of the above reactions was investigated. Chiral analysis of the 2-alkanols in reactions (1) to (3) showed that the alkanols produced in these reactions were racemic mixtures (equal mole fractions) of both the (*S*)-(+)-2-alkanol and the (*R*)-(−)-2-alkanol. However, reaction (4) had a much greater degree of stereoselectivity and the product was primarily the S-(+)-2-heptanol. For reaction (5), the only product was (*S*)-(+)-2-octanol.

2. Experimental

2.1. Materials

The substances used in this study, their Chemical Abstract Service (CAS) registry numbers, empirical formulas, molar masses, sources, and purities as determined by gas chromatography (g.c.) are given in table 1. [1] The enzyme used in this study was ketoreductase (EC 1.1.1.2) from Biocatalytics, Inc., Pasadena, CA. This enzyme was prepared from a recombinant bacterial source and gene expressed in *Escherichia coli*; {NADP(red)} was embedded in the ketoreductase preparation by the vendor.

2.2. Chromatography and quantitative analysis

The quantitative analysis of the reactants and products was carried out by using a Hewlett-Packard (HP) 5890 g.c. (Agilent Technologies, Wilmington, DE, USA), equipped with a flame ionization detector (FID) and a fused silica Phenomenex ZB-FFAP capillary column (30 m long, 0.53 mm id, 0.53 μm thick film coating). The injector and detector temperatures were $T = 523$ K and $T = 543$ K, respectively; the head pressure of the helium carrier gas was $P = 283$ kPa. The initial column temperature of $T = 313$ K was held for 3 min and then raised to $T = 513$ K at a rate of 0.333 $K \cdot s^{-1}$ and held at $T = 513$ K for 5 min.

Enantioselective separation of the (*R*)-(−)-2-alkanols and (*S*)-(+)-2-alkanols was carried out with another HP 5890 g.c. equipped with a FID and a fused silica γ-cyclodextrin trifluoroacetyl column (30 m long, 0.25 mm i.d., Chiraldex, Astec, Whippany, NJ, USA). For all of the alkanols, the helium carrier gas flow was held constant at 0.017 $cm^3 \cdot s^{-1}$ with a detector make-up flow of 0.50 $cm^3 \cdot s^{-1}$. The injector and detector were held at $T = 423$ K. All injections were split with a split flow rate of the alkanols, the g.c. oven condition varied with the alkanol. The butanols were not run on this column because the temperature needed to separate the enantiomers was lower than could be obtained with the apparatus. For the separation of the 2-pentanols, the oven was held at $T = 305$ K for 20 min and then raised to $T = 313$ K at a rate of 0.0167 $K \cdot s^{-1}$ and then held at $T = 313$ K for 2.5 min. For the separation of the 2-hexanols, the oven was held at $T = 308$ K for 15 min and then raised to $T = 333$ K at a rate of 0.0334 $K \cdot s^{-1}$ and held at $T = 333$ K for 2.5 min. For the separation of the 2-heptanols, the oven was held at $T = 308$ K for 15 min and then raised to $T = 333$ K at a rate of 0.0167 $K \cdot s^{-1}$ and held at $T = 333$ K for 5 min. For the separation of the 2-octanols, the oven was held isothermally at $T = 323$ K for 50 min.

Internal standards were used for the quantitative determination of the concentrations of the substances in reactions (1) to (5). The internal standards were: (*S*)-(+)-2-octanol for reaction (1) carried out in *n*-hexane, $SCCO_2$, and MTBE; 1-hexanol for reaction (1) carried out in toluene; 2-octanol for reactions (2)–(4) carried out in *n*-hexane; 1-butanol for reaction (5) carried out in *n*-hexane and in MTBE; and 1-hexanol for reaction (5) carried out in toluene. By using the chromatographic conditions described above, the chromatographic peaks of the compounds of interest and their respective internal standards were well separated.

For the quantitative analysis of the reactants and products in the equilibrated reaction mixtures in *n*-hexane, toluene and MTBE, ≈4 cm^3 of reaction mixture and ≈0.05 cm^3 of internal standard solution were gravimetrically added to a vial, and tightly capped. Approximately $6 \cdot 10^{-4}$ cm^3 of this solution was then injected into the g.c., and the reaction mixture was analyzed for reactants and products. The concentrations *c* {expressed as mol $(kg \cdot sln)^{-1}$} of each of the substances involved in the reactions were determined from their respective chromatographic peak areas, the appropriate response factor ratios, the concentration of the internal standard solution, and the chromatographic peak area of the internal standard.

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

TABLE 1
Principal substances used in this study with their Chemical Abstracts Service (CAS) registry numbers, empirical formulas, and molecular masses M_r

Substance	CAS no.	Formula	M_r	x_1	x_2
Acetone	67-64-1	C_3H_6O	58.08	0.99	
(*R*)-(−)-2-Butanol	14898-79-4	$C_4H_{10}O$	74.12	0.99	0.99
(*S*)-(+)-2-Butanol	4221-99-2	$C_4H_{10}O$	74.12	0.99	0.99
2-Butanone	78-93-3	C_4H_8O	72.11	0.99	
(*R*)-(−)-2-Heptanol	6033-24-5	$C_7H_{16}O$	116.20	0.96	0.96
(*S*)-(+)-2-Heptanol	6033-23-4	$C_7H_{16}O$	116.20	0.99	0.99
2-Heptanone	110-43-0	$C_7H_{14}O$	114.19	0.99	
n-Hexane	110-54-3	C_6H_{14}	86.18	0.995	
(*R*)-(−)-2-Hexanol	26549-24-6	$C_6H_{14}O$	102.18	0.99	0.99
(*S*)-(+)-2-Hexanol	52019-78-0	$C_6H_{14}O$	102.18	0.99	0.99
2-Hexanone	591-78-6	$C_6H_{12}O$	100.16	0.98	
Methyl *tert*-butyl ether	1634-04-4	$C_5H_{12}O$	88.15	0.998	
(*R*)-(−)-2-Octanol	5978-70-1	$C_8H_{18}O_2$	130.23	0.98	0.98
(*S*)-(+)-2-Octanol	6169-06-8	$C_8H_{18}O_2$	130.23	0.99	0.99
2-Octanone	111-13-7	$C_8H_{16}O_2$	128.22	0.98	
(*R*)-(−)-2-Pentanol	31087-44-2	$C_5H_{12}O$	88.15	0.99	0.99
(*S*)-(+)-2-Pentanol	26184-62-3	$C_5H_{12}O$	88.15	0.99	0.99
2-Pentanone	107-87-9	$C_5H_{10}O$	86.13	0.995	
2-Propanol	67-63-0	C_3H_8O	60.10	0.998	
Toluene	108-88-3	C_7H_8	92.14	0.9999	

All of these substances were obtained from Aldrich with the exception of toluene which was Reference Material NIMC-CRM-4003 from the National Institute of Materials and Chemical Research of Japan. The total mole fraction purities x_1 are those determined by the vendor by using g.c. The chiral mole fraction purities x_2 of the alcohols were determined in our laboratory (see Section 2.2).

2.3. Equilibrium studies

The equilibrium measurements were carried out by approaching equilibrium from both directions of reaction. Solutions consisting of {2-alkanone + 2-propanol} were used for the forward direction and solutions of {(*S*)-(+)-2-alkanol + acetone} were used for the reverse direction. The substrates were first dissolved in the solvents *n*-hexane, MTBE, and toluene. The total volume of the solutions used was ≈15 cm^3. Then, 0.10 cm^3 of a solution containing the enzyme [0.010 g of ketoreductase dissolved in 0.10 cm^3 of phosphate buffer {K_2HPO_4 (c = 0.10 mol · dm^3), adjusted to pH 7.3 with H_3PO_4}] was added to each reaction mixture. These reaction mixtures were then placed in a constant temperature bath (±0.01 K) and shaken laterally at ≈30 shakes · min^{-1}. The solutions were periodically analyzed to determine the extent of reaction. Reactions were considered to be at equilibrium when the ratios of the g.c. peak area ratios of products:reactants were essentially identical for the forward and the reverse reaction mixtures. Additional enzyme solution was added to the reaction mixtures if it was found necessary to speed the approach to equilibrium. Reaction (1) equilibrated in 4 h for all three solvents. However, reactions (2) and (3) required, respectively, 1 day and 3 days to reach equilibrium. Reactions (4) and (5) required additional enzyme and took ≈7 days to reach equilibrium.

For the equilibrium study in $SCCO_2$, a stainless steel reaction vessel (volume = 15 cm^3) was used [19]. As described previously [19], a HP supercritical fluid chromatograph (SFC, Agilent Technologies, Wilmington, DE, USA) with a modified three-port valve was used to connect the reaction vessel in the system.

For the measurement of the equilibrium constant for reaction (1) in $SCCO_2$, ≈0.20 g of an equimolar solution of (2-butanone + 2-propanol) was first prepared and placed in the stainless steel reaction vessel. Then 0.10 cm^3 of a solution containing the enzyme [0.010 g of ketoreductase dissolved in 0.10 cm^3 of phosphate buffer {K_2HPO_4 (c = 0.10 mol · dm^3), adjusted to pH 7.3 with H_3PO_4}] was added to the reaction vessel. The reaction vessel was then assembled and connected to the SFC system. The reaction was allowed to proceed for five hours in the $SCCO_2$ media at T = 303.15 K and P = 10.0 MPa. During the course of the reaction, the vessel was gently shaken at approximately 15 min intervals. After completion of reaction, the vessel was depressurized by opening the needle valve while bubbling CO_2 (g) into a 20 cm^3 volumetric flask containing ≈7 cm^3 of *n*-hexane in order to collect the escaping reactants and products. Once the pressure in the vessel reached $p \approx 0.1$ MPa, the vessel was disconnected from the SFC system, and the cover of the reaction vessel was removed. The *n*-hexane in the volumetric flask was poured into the reaction vessel and an additional quantity of *n*-hexane was added to the reaction vessel to fill it completely. Then, ≈0.50 cm^3 of this diluted reaction mixture, ≈0.10 cm^3 of the internal standard solution, and ≈10 cm^3 of *n*-hexane were gravimetrically added to a vial. Analysis for the concentrations of acetone, 2-propanol, 2-butanone and 2-butanol was done by using the first g.c. method (FID with ZB-FFAP col-

umn) described above. For the measurement of the equilibrium constants from the reverse direction, the reactions were carried out in the same way except that the starting mixtures were, respectively, equimolar mixtures of {(*S*)-(+)-2-butanol + acetone} and of {(*S*)-(+)-2-pentanol + acetone}.

3. Results and discussion

3.1. Thermodynamic formalism

The respective equilibrium constants for reactions (1) through (5) are:

$$K = c(\text{2-butanol}) \cdot c(\text{acetone}) / \{c(\text{2-butanone}) \cdot c(\text{2-propanol})\}, \quad (9)$$

$$K = c(\text{2-pentanol}) \cdot c(\text{acetone}) / \{c(\text{2-pentanone}) \cdot c(\text{2-propanol})\}, \quad (10)$$

$$K = c(\text{2-hexanol}) \cdot c(\text{acetone}) / \{c(\text{2-hexanone}) \cdot c(\text{2-propanol})\}, \quad (11)$$

$$K = c(\text{2-heptanol}) \cdot c(\text{acetone}) / \{c(\text{2-heptanone}) \cdot c(\text{2-propanol})\}, \quad (12)$$

$$K = c(\text{2-octanol}) \cdot c(\text{acetone}) / \{c(\text{2-octanone}) \cdot c(\text{2-propanol})\}. \quad (13)$$

Here, c is the concentration of the indicated substance. In organic solvents, the substrates involved in these reactions are in a non-ionized form and their concentrations are small. Hence, we have assumed that their activity coefficients are unity and that the calculated equilibrium constants can be identified as thermodynamic equilibrium constants defined in terms of activities of the reactants and the products.

It is also possible to consider reactions (1) to (5) in terms of the formation of a specific chiral product. This generic reaction, written in terms of the (*S*)-(+) isomer, is

$$\text{2-alkanone} + \text{2-propanol} = (S)\text{-}(+)\text{-2-alkanol} + \text{acetone}. \quad (14)$$

The equilibrium constant for this reaction is

$$K = c\{(S)\text{-}(+)\text{-2-alkanol}\} \cdot c(\text{acetone}) / \{c(\text{2-alkanone}) \cdot c(\text{2-propanol})\}. \quad (15)$$

The equilibrium constant for the generic reaction {see reaction (8)} involving the racemic mixture {equal amounts of (*R*) and (*S*) isomers} is

$$K = c(\text{2-alkanol}) \cdot c(\text{acetone}) / \{c(\text{2-alkanone}) \cdot c(\text{2-propanol})\}, \quad (16)$$

where $c(\text{2-alkanol}) = c\{(R)\text{-}(+)\text{-2-alkanol}\} + c\{(S)\text{-}(+)\text{-2-alkanol}\}$. By using the relationships $c\{(R)\text{-}(+)\text{-2-alkanol}\} = c\{(S)\text{-}(+)\text{-2-alkanol}\}$,

$$c(\text{2-alkanol}) = 2 \cdot c\{(R)\text{-}(+)\text{-2-alkanol}\} = 2 \cdot c\{(S)\text{-}(+)\text{-2-alkanol}\}, \quad (17)$$

one obtains

$$K(8) = 2 \cdot K(14). \quad (18)$$

In the treatment of the results and in the discussion to follow, we shall deal solely with equilibrium constants for reactions (1) to (5) and that are defined by equations (9) to (13) and where the total amount of 2-alkanol is considered. The equilibrium constants that pertain to reactions {see equation (14)} involving specific chiral products can be calculated easily by using equation (18).

3.2. Results of equilibrium measurements

The results of the equilibrium measurements for the ketoreductase-catalyzed reduction of 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-octanone to the corresponding 2-alkanols in the several solvents used in this study are given in table 2. Attempts were also made to use $SCCO_2$ as a solvent for reactions that use 2-hexanone, 2-heptanone, and 2-octanone. However, the rates of reaction for these substances in this solvent were not rapid enough to conveniently carry out measurements of the desired equilibrium constants. It is important to note that the values of the equilibrium constants obtained from the forward and the reverse directions of reaction are either in agreement or very close to agreement within their statistical uncertainties. Thus, the reactions are judged to have reached equilibrium in terms of the total amounts of the substances shown in reactions (1) to (5). The values of the equilibrium constants $\langle K \rangle$ reported in the last column in table 2 are the averages of the results obtained from the forward and the reverse directions of reaction. The uncertainties given in column 8 of table 2 are based on the random errors in the measurements expressed as two estimated standard deviations of the mean and do not include possible systematic errors in the measurements. It is judged that reasonable estimates of the standard uncertainties [23] due to possible systematic errors in the values of the equilibrium constants for these reactions are: $\pm 0.03 \cdot K$ in the g.c. measurements of the concentrations of the reactants and products (this includes possible errors in the values of the response factors) and $\pm 0.01 \cdot K$ due to possible sample impurities. These estimated uncertainties have been combined in quadrature together with the statistical uncertainties, expressed as one estimated standard deviation of the mean, to obtain

TABLE 2
Results of equilibrium measurements of the ketoreductase-catalyzed reduction of 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-octanone to the corresponding 2-alkanols in several solvents {reactions (1) to (5)}

Solvent	Direction	T/K	c(2-alkanone) / mol · (kg · soln)$^{-1}$	c(2-propanol) / mol · (kg · soln)$^{-1}$	c(2-alkanol) / mol · (kg · soln)$^{-1}$	c(acetone) / mol · (kg · soln)$^{-1}$	K	$\langle K \rangle$
2-Butanone(soln) + 2-propanol(soln) = 2-butanol(soln) + acetone(soln) (1)								
n-Hexane	Forward	288.15	0.01117	0.00499	0.00557	0.00844	0.843 ± 0.009	0.845 ± 0.054
	Reverse	288.15	0.01129	0.00521	0.00483	0.01031	0.847 ± 0.005	
	Forward	293.05	0.01106	0.00635	0.00630	0.00940	0.843 ± 0.003	0.844 ± 0.055
	Reverse	293.05	0.01126	0.00658	0.00552	0.01133	0.844 ± 0.005	
	Forward	298.15	0.01097	0.00604	0.00671	0.00837	0.848 ± 0.003	0.844 ± 0.054
	Reverse	298.15	0.01109	0.00657	0.00503	0.01217	0.840 ± 0.004	
	Forward	303.30	0.01107	0.00690	0.00658	0.00986	0.849 ± 0.004	0.849 ± 0.054
	Reverse	303.30	0.01129	0.00728	0.00578	0.01208	0.850 ± 0.003	
	Forward	308.27	0.01095	0.00821	0.00933	0.00777	0.806 ± 0.007	0.808 ± 0.052
	Reverse	308.27	0.00992	0.00706	0.00855	0.00663	0.809 ± 0.008	
Toluene	Forward	298.15	0.01198	0.01064	0.01025	0.01055	0.848 ± 0.011	0.853 ± 0.056
	Reverse	298.15	0.01391	0.01009	0.01039	0.01157	0.857 ± 0.014	
$SCCO_2$	Forward	303.15	0.00570	0.00355	0.00681	0.00242	0.814 ± 0.011	0.820 ± 0.054
	Reverse	303.15	0.01313	0.00859	0.01077	0.00865	0.826 ± 0.014	
MTBE	Forward	298.15	0.01038	0.01002	0.00935	0.00909	0.817 ± 0.007	0.814 ± 0.052
	Reverse	298.15	0.00798	0.00653	0.00793	0.00533	0.811 ± 0.006	
2-Pentanone(soln) + 2-propanol(soln) = 2-pentanol(soln) + acetone(soln) (2)								
n-Hexane	Forward	298.15	0.00572	0.00584	0.00433	0.00483	0.626 ± 0.003	0.627 ± 0.040
	Reverse	298.15	0.00677	0.00772	0.00523	0.00629	0.629 ± 0.002	
$SCCO_2$	Forward	303.15	0.02318	0.01394	0.02534	0.00796	0.624 ± 0.008	0.634 ± 0.041
	Reverse	303.15	0.02848	0.01622	0.04951	0.00601	0.644 ± 0.010	
2-Hexanone(soln) + 2-propanol(soln) = 2-hexanol(soln) + acetone(soln) (3)								
n-Hexane	Forward	298.15	0.00917	0.00674	0.00333	0.00846	0.456 ± 0.003	0.459 ± 0.029
	Reverse	298.15	0.00900	0.00650	0.00315	0.00856	0.461 ± 0.005	
2-Heptanone(soln) + 2-propanol(soln) = 2-heptanol(soln) + acetone(soln) (4)								
n-Hexane	Forward	298.15	0.00872	0.00532	0.00271	0.00576	0.336 ± 0.005	0.338 ± 0.022
	Reverse	298.15	0.00849	0.00561	0.00279	0.00580	0.340 ± 0.002	
2-Octanone(soln) + 2-propanol(soln) = 2-octanol(soln) + acetone(soln) (5)								
n-Hexane	Forward	298.15	0.00558	0.00695	0.00343	0.00351	0.310 ± 0.005	0.313 ± 0.020
	Reverse	298.15	0.00855	0.00405	0.00110	0.00992	0.315 ± 0.001	
Toluene	Forward	298.15	0.00442	0.00533	0.00141	0.00520	0.311 ± 0.007	0.311 ± 0.021
	Reverse	298.15	0.00451	0.00545	0.00129	0.00592	0.311 ± 0.007	
MTBE	Forward	298.15	0.00434	0.01172	0.00326	0.00488	0.313 ± 0.003	0.313 ± 0.020
	Reverse	298.15	0.00439	0.01139	0.00322	0.00488	0.314 ± 0.002	

Abbreviations are: MTBE, methyl *tert*-butyl ether; and $SCCO_2$, supercritical carbon dioxide (P = 10.0 MPa). The concentrations of the substrates involved in the reaction {2-alkanone(soln) + 2-propanol(soln) = 2-alkanol(soln) + acetone(soln)} are given in columns 4 to 7. The 2-butanol, 2-pentanol, and 2-hexanol are racemic mixtures {equal amounts of the respective (*R*) and (*S*) isomers}. The 2-heptanol is a mixture of (*S*)-(+)-2-heptanol and (*R*)-(−)-2-heptanol, at the respective mole fractions of 0.95 and 0.05. The 2-octanol consists solely of (*S*)-(+)-2-octanol. Each concentration is the average of five measurements. The values of the equilibrium constants K (column 8) are calculated from the concentrations given in columns 4 to 7 and by using equations (9) to (13). The quantity $\langle K \rangle$ is the average of the equilibrium constants which were measured from the forward and the reverse directions of reaction. The uncertainties in the values of K (column 8) are based on two estimated standard deviations of the mean. The uncertainties in the values of $\langle K \rangle$ (column 9) are combined expanded uncertainties (see Section 3.2).

combined standard uncertainties [23]. These combined uncertainties are then multiplied by two to obtain the expanded uncertainties given in the average values of K (see column 9 in table 2).

Chiral analysis of the reaction products showed that the enzyme used in this study was stereoselective for the 2-octanone system – only (*S*)-(+)-2-octanol was formed. For the reactions involving butanone, pentanone, and hexanone, the products were racemic mixtures of the respective (*S*)-(+)-2-alkanol and the (*R*)-(−)-2-alkanol. Chiral analysis showed that for the 2-heptanone system reaction (4), the 2-alkanol product was a mixture of (*S*)-(+)-2-heptanol and (*R*)-(−)-2-heptanol, at the respective mole fractions of 0.95 and 0.05.

A comparison of the values of the equilibrium constants for reaction (1) in *n*-hexane, toluene, MTBE, and $SCCO_2$ shows that these values have a small dependence ($<0.036 \cdot K$) on the solvent used to carry out the reaction. Similarly, the values of the equilibrium constants for reaction (2) in *n*-hexane and $SCCO_2$ and for reaction (5) in *n*-hexane, toluene, and MTBE do not vary with the solvent. This result has potential implications for process design. Specifically, since the theoretical product yields for these reactions do not vary significantly with the solvent, one can select the solvent based on kinetic, economic, environmental, or separation considerations.

We have calculated [24] values of the standard molar Gibbs free energy change $\Delta_r G^\circ_m$, the standard molar enthalpy change $\Delta_r H^\circ_m$, and the standard molar entropy change $\Delta_r S^\circ_m$ from the temperature dependency of the equilibrium constant for reaction (1) carried out in *n*-hexane. In performing this calculation, it was assumed that that the standard molar heat capacity change $\Delta_r C^\circ_m$, for this reaction was zero. The calculated values for these thermodynamic quantities at $T = 298.15$ K are: $K = 0.838 \pm 0.013$; $\Delta_r G^\circ_m = (0.44 \pm 0.040)$ kJ $\cdot$ mol^{-1}; $\Delta_r H^\circ_m = -(1.2 \pm 1.7)$ kJ $\cdot$ mol^{-1}; and $\Delta_r S^\circ_m = -(5.5 \pm 5.7)$ J $\cdot$ K^{-1} $\cdot$ mol^{-1}. These uncertainties are equal to two estimated standard deviations of the mean.

Shown in figure 2 is a plot of the equilibrium constants for reactions (1) to (5) carried out in *n*-hexane as a function of the number of carbons N_C in the 2-alkanone. The values of the equilibrium constants decrease monotonically with increasing value of N_C and trend towards a limiting value of ≈ 0.30 for $N_C > 8$.

The ketoreductase used in this study has been reported to be stereoselective [12] for the production of (*S*)-(+)-2-octanol from 2-octanone. This result was confirmed in the chiral analysis performed in this study. However, in our study we also found that reduction of 2-heptanone produced 0.95 mole fraction of (*S*)-(+)-2-heptanol and 0.05 mole fraction of (*R*)-(−)-2-heptanol while shorter chain length 2-alkanones produced racemic mixtures of both (*S*)-(+)-2-alkanol and (*R*)-(−)-2-alkanol. Clearly, these findings have implications for understanding the interaction of the 2-octanone with the active site of the ketoreductase enzyme and for the mechanism of these reactions. In any case, if one wishes to produce optically active alkanols, there are additional enzymes that might be utilized. Specifically, Kruse *et al.* [8] used alcohol dehydrogenase coupled with formate dehydrogenase for the production of chiral (*S*)-(+)-alcohols from the corresponding ketones. Also, Nanduri *et al.* [10] have recently purified a NAD(red) dependent ketoreductase from *Gluconobactor Oxydans* with a high enantioselectivity. They [10] used this enzyme, coupled with formate dehydrogenase, for the stereoselective production of (*S*)-(+)-2-pentanol from 2-pentanone. Having the thermodynamic results in place for the reactions studied herein and for additional representative reactions can play an important role in the engineering of a process used to make the desired products in bulk and with a high product yield. The results obtained herein are believed to be the first thermodynamic results obtained on these reactions.

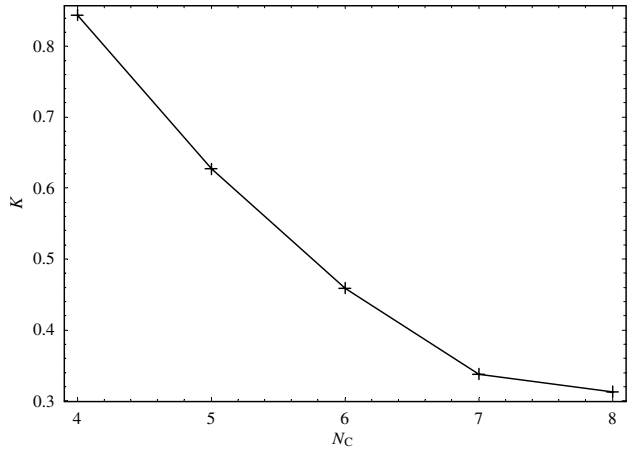

FIGURE 2. The equilibrium constants K for reactions (1) to (5) plotted against the number of carbons N_C in the alkanone. The solvent was *n*-hexane and the temperature was 298.15 K.

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