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A thermodynamic study of ketoreductase-catalyzed reactions 3. Reduction of 1-phenyl-1-alkanones in non-aqueous solvents

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Abstract

The equilibrium constants K for the reactions (1-phenyl-1-alkanone + 2-propanol = 1-phenyl-1-alkanol + acetone) in the solvents *n*-pentane and *n*-hexane have been determined by using gas chromatography at the temperature 298.15 K. The 1-phenyl-1-alkaones included in this study were: 1-phenyl-1-ethanone, 1-phenyl-1-propanone, 1-phenyl-1-butanone, 1-phenyl-1-pentanone, 1-phenyl-1-hexanone, and 1-phenyl-1-heptanone. The equilibrium constants for the reaction involving 1-phenyl-1-ethanone were measured in the solvent *n*-hexane as a function of temperature (288 K to 308 K). The calculated thermodynamic quantities for the 1-phenyl-1-ethanone reaction at $T = 298.15$ K are: $K = 0.2177 \pm 0.0018$; the standard molar Gibbs free energy change, $\Delta_r G^\circ_m = (3.78 \pm 0.02)$ kJ · mol^{-1}, the standard molar enthalpy change, $\Delta_r H^\circ_m = (4.53 \pm 0.87)$ kJ · mol^{-1}, and the standard molar entropy change, $\Delta_r S^\circ_m = (2.5 \pm 2.9)$ J · K^{-1} · mol^{-1}. The equilibrium constants of 1-phenyl-1-alkanone with an odd number of carbons in alkyl side chain are higher than the equilibrium constants of the preceding 1-phenyl-1-alkanone having an even number of carbons in the side chain and follow a zig-zag pattern with increasing carbon number in the alkyl side chain.
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1. Introduction

In recent years, the use of biocatalysis for organic synthesis [1,2] in non-aqueous media has become an attractive alternative to traditional chemical synthetic methods. The use of enzyme-catalyzed reactions has made it possible to obtain desired stereoselective products which are useful intermediates in the pharmaceutical, agrochemical, and perfume industries [3–5]. Lipase-catalyzed reactions in organic solvents have been used for the stereoselective resolution of racemic mixtures [4,6–8]. Ketoreductase catalyzed reduction of carbonyl groups [9–16] have been used for the synthesis of chiral secondary alcohols which are useful intermediates for pharmaceuticals, agrochemicals, and liquid crystals.

There have been several studies from our laboratory dealing with the thermodynamics of enzyme-catalyzed reactions in organic solvents [17–22]. 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 enzyme-catalyzed reduction reactions of 1-phenyl-1-alkanone in non-aqueous solvents.

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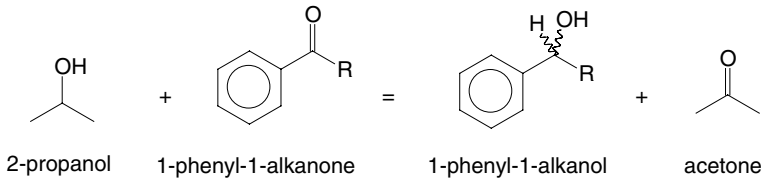

FIGURE 1. The structures of the substances involved in the reduction of 1-phenyl-1-alkanones {reactions (1) to (6)}, where R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, or C_6H_{13}.

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

1-phenyl-1-ethanone(soln) + 2-propanol(soln) = 1-phenyl-1-ethanol(soln) + acetone(soln), (1)

1-phenyl-1-propanone(soln) + 2-propanol(soln) = 1-phenyl-1-propanol(soln) + acetone(soln), (2)

1-phenyl-1-butanone(soln) + 2-propanol(soln) = 1-phenyl-1-butanol(soln) + acetone(soln), (3)

1-phenyl-1-pentanone(soln) + 2-propanol(soln) = 1-phenyl-1-pentanol(soln) + acetone(soln), (4)

1-phenyl-1-hexanone(soln) + 2-propanol(soln) = 1-phenyl-1-hexanol(soln) + acetone(soln), (5)

1-phenyl-1-heptanone(soln) + 2-propanol(soln) = 1-phenyl-1-heptanol(soln) + acetone(soln). (6)

Here "soln" denotes the organic solvent, *n*-pentane or *n*-hexane, have been used in this study.

A small amount of β-nicotinamide-adenine dinucleotide (reduced), {NAD(red)} is required for catalytic activity of this ketoreductase. As described in our previous papers [21,22], the ketoreductase catalyzed reactions proceed in two steps. In the first step, the 1-phenyl-1-alkanone is reduced to the corresponding 1-phenyl-1-alkanol and NAD(ox). Then in the second step, the NAD(red) is regenerated by reduction of NAD(ox) in presence of 2-propanol:

1-phenyl-1-alkanone(soln) + 2NAD(red) = 1-phenyl-1-alkanol(soln) + 2NAD(ox) (7)

2NAD(ox) + 2-propanol(soln) = 2NAD(red) + acetone(soln). (8)

Thus, the combination of equations (7) and (8) leads to an overall reaction for the reduction of the 1-phenyl-1-alkanone:

1-phenyl-1-alkanone(soln) + 2-propanol(soln) = 1-phenyl-1-alkanol(soln) + acetone(soln). (9)

The principal aim of this study was to determine the equilibrium constants for the above 1-phenyl-1-alkanone reactions as a function of the number of carbons in the alkyl chain. The chirality of these reactions has also been investigated. The chiral analysis of the 1-phenyl-1-alkanols in reactions (1) to (6) showed that the 1-phenyl-1-alkanols produced in these reduction reactions were racemic mixtures (equal mole fractions) of both the (*R*)-(+)-1-phenyl-1-alkanol and the (*S*)-(−)-1-phenyl-1-alkanol.

2. Experimental

2.1. Materials

The substances used in this study, their Chemical Abstract Service (CAS) registry numbers, empirical formulae, molar masses, sources, and purities as determined by gas chromatography (g.c.) are given in table 1. The mass fraction of water listed in table 1 for the compounds involved in these reactions and the internal standard, 1-hexanol were determined by Karl Fischer titration [23]. 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 *E. coli* and required NAD(red) catalytic activities.

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),[1] equipped with a flame ionization detector (FID) and a fused silica Phenomenex ZB-FFAP capillary column (30 m long, 0.53 mm i.d., 0.53 μm thick film coating). The injector and detector temperatures were 523 K and 543 K, respectively; the head pressure of the helium carrier gas was $P = 283$ kPa. The initial column temperature of 313 K was held for 3 min and then raised to $T = 513$ K at a rate of $0.333\ \mathrm{K \cdot s^{-1}}$ and held at $T = 513$ K for 5 min. Under these conditions, the chromatographic peaks of the compounds of interest and the internal standard were well separated.

Enantioselective separation of the (*R*)-(+)-1-phenyl-1-alkanol and (*S*)-(−)-1-phenyl-1-alkanol was carried out with another HP 5890 g.c. equipped with an FID and a fused silica γ-cyclodextrintrifluoroacetyl capillary column

[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, relative molecular masses M_r, Supplier (A, Aldrich; B, BioCatalytics; R, Roche; S, synthesized; and T, TCI America), mole fraction purity x as stated by the vendor, mass fraction moisture contents w determined by Karl Fischer analysis, x(chiral) is the chiral mole fraction purity of alcohols as stated by vendors, r designates racemic mixture, and the methods used to determine the mole fraction purity and chiral purity

Substance	CAS no.	Formula	M_r	Supplier	x	w	x (chiral)	Method
Acetone	67-64-1	C_3H_6O	58.08	A	0.99	0.0103		g.c.
n-Hexane	110-54-3	C_6H_{14}	86.18	A	0.99			g.c.
1-Hexanol	111-27-3	$C_6H_{14}O$	102.18	A	0.99	0.0011		g.c.
Ketoreductase (EC 1.1.1.2)				B				
NAD(red)[a]	24292-60-2	$C_{21}H_{26}N_7Na_2P_3O_{17}$	787.4	R	0.97			Enzymatic assay
n-Pentane	109-66-0	C_5H_{12}	72.15	A	0.99			g.c.
(*R*)-(+)-1-Phenyl-1-butanol	22144-60-1	$C_{10}H_{14}O$	150.22	A	0.97	0.0018	0.98	FT-n.m.r., g.c.
(*S*)-(−)-1-Phenyl-1-butanol	22135-49-5	$C_{10}H_{14}O$	150.22	A	0.97	0.0060	0.99	FT-n.m.r., g.c.
1-Phenyl-1-butanone (butyrophenone)	495-40-9	$C_{10}H_{12}O$	148.21	A	0.99	0.0036		FT-n.m.r., FT-IR, g.c.
(*R*)-(+)-1-Phenyl ethanol	1517-69-7	$C_8H_{10}O$	122.17	A	0.99	0.0066	0.99	g.c.
(*S*)-(−)-1-Phenyl ethanol	1445-91-6	$C_8H_{10}O$	122.17	A	0.99	0.0047	0.98	g.c.
1-Phenyl-1-ethanone-1 (acetophenone)	98-86-2	C_8H_8O	120.15	A	0.99	0.0026		FT-n.m.r., FT-IR, g.c.
1-Phenyl-1-heptanol		$C_{13}H_{20}O$	192.29	S	0.99	0.0009	*r*	g.c., n.m.r.
1-Phenyl-1-heptanone (*n*-heptanophenone)	1671-75-6	$C_{13}H_{18}O$	190.28	*T*	0.98	0.0019		g.c.
1-Phenyl-1-hexanol		$C_{12}H_{18}O$	178.26	S	0.99	0.0008	*r*	g.c., n.m.r.
1-Phenyl-1-hexanone (*n*-hexanophenone)	942-92-7	$C_{12}H_{16}O$	176.25	*T*	0.98	0.0010		g.c.
1-Phenyl-1-pentanol	583-03-9	$C_{11}H_{16}O$	164.24	T	0.99	0.0025	*r*	g.c.
1-Phenyl-1-pentanone (valerophenone)	1009-14-9	$C_{11}H_{14}O$	162.23	*T*	0.995	0.0016		FT-n.m.r., g.c.
(*R*)-(+)-1-Phenyl-1-propanol	1565-74-8	$C_9H_{12}O$	136.19	A	0.99	0.0029	0.99	g.c.
(*S*)-(−)-1-Phenyl-1-propanol	613-87-6	$C_9H_{12}O$	136.19	A	0.99	0.0015	0.99	g.c.
1-Phenyl-1-propanone (propiophenone)	93-55-0	$C_9H_{10}O$	134.18	A	0.99	0.0024		FT-n.m.r., FT-IR
2-Propanol	67-63-0	C_3H_8O	60.10	A	0.995	0.0006		FT-n.m.r., FT-IR

Some common names of the substances are given in parentheses.
[a] NAD(red) is the reduced form of β-nicotinamide-adenine dinucleotide phosphate, disodium salt.

(30 m long, 0.25 mm i.d., Chiraldex, Astec, Whippany, NJ, USA). For analysis of (*R*) and (*S*) isomers of 1-phenyl-1-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 $0.50\ cm^3 \cdot s^{-1}$. For the separation of (*R*) and (*S*) isomers of 1-phenyl ethanol through 1-phenyl-1-butanol in reactions (1) to (3), the oven was held at $T = 303$ K for 15 min followed by a temperature ramp of $0.167\ K \cdot s^{-1}$ to 423 K, where the oven was held for 10 min. For the separation of the (*R*) and (*S*) isomers of 1-phenyl-1-pentanol in reaction (4), the oven was held isothermally at $T = 393$ K for 40 min. For the separation of the (*R*) and (*S*) isomers of 1-phenyl-1-hexanol in reaction (5) and the (*R*) and (*S*) isomers of 1-phenyl-1-heptanol in reaction (6), the oven was held at $T = 373$ K for 20 min followed by a temperature ramp of $0.067\ K \cdot s^{-1}$ to $T = 473$ K, where the oven was held for 13 min.

2.3. Synthesis of 1-phenyl-1-hexanol and 1-phenyl-1-heptanol

First, 0.05 mol of *n*-hexanophenone or *n*-heptanophenone was dissolved in $100\ cm^3$ of methanol maintained at $T \approx 288$ K. Another solution containing $n = 0.018$ mol of $NaBH_4$ dissolved in $1\ cm^3$ of NaOH ($c = 2.0\ mol \cdot dm^{-3}$) and then diluted with $9\ cm^3$ of water was also prepared [24]. The $NaBH_4$ solution was slowly added to *n*-hexanophenone or *n*-heptanophenone solution so that the temperature of the mixture was maintained at $T \approx 293$ K. This reaction mixture was gently stirred overnight under reduced pressure at room temperature (≈293 K). The methanol was removed from the reaction mixture and $\approx 50\ cm^3$ of water was added to the residue. The product was then extracted twice into $50\ cm^3$ diethyl ether. The ether containing the 1-phenyl-1-alkanol was dried over $MgSO_4$ and removed in vacuo.

The product yield (mole fraction) of crude 1-phenyl-1-alkanol product was >0.90. The 1-phenyl-1-alkanol product was further purified by vacuum distillation at $T \approx 293$ K and at $P = 120$ Pa for 1-phenyl-1-hexanol, and at $T \approx 377$ K and at $P = 66.7$ Pa for 1-phenyl-1-heptanol. The purity of these 1-phenyl-1-alkanols was then checked by using ^1H n.m.r. and gas chromatography. The chirality check on an enantioselective γ-cyclodextrintrifluoroacetyl capillary g.c. column showed that the 1-phenyl-1-hexanol was a racemic mixture of (*R*)-(+)-1-phenyl-1-hexanol and (*S*)-(−)- 1-phenyl-1-hexanol, and the 1-phenyl-1-heptanol was also a racemic mixture of (*R*)-(+)-1-phenyl-1-heptanol and the (*S*)-(−)-1-phenyl-1-heptanol.

2.4. Response factor ratios

As described previously [22], a standard solution of acetone, 1-phenyl-1-ethanone, 1-phenyl-1-ethanol, 2-propanol, and 1-hexanol was prepared gravimetrically in *n*-hexane. Using this solution the response factors (ratio of concentration to peak area) with reference to the internal standard, 1-hexanol, were determined for acetone, 1-phenyl-

1-ethanone, 1-phenyl-1- ethanol, and 2-propanol. For reactions (2) to (6) standard solutions of 1-phenyl-1-alkanone, its 1-phenyl-1-alkanol, and 1-hexanol were prepared in *n*-hexane and their response factor ratios with respect to 1-hexanol were determined. Similarly, for the reactions in *n*-pentane as solvent, a solution of acetone, 2-propanol, and 1-hexanol was gravimetrically prepared in *n*-pentane, and the response factor ratios of acetone and 2-propanol with respect to 1-hexanol [22] were determined in *n*-pentane.

2.5. Equilibrium studies

The equilibrium measurements were carried out by approaching equilibrium from both directions of reaction. For the forward direction, the starting solution consisted of {1-phenyl-1-alkanone + 2-propanol}, and for the reverse direction, the starting solutions were {1-phenyl-1-alkanol + acetone}. The substrates were first dissolved in $\approx 15\ \mathrm{cm}^3$ of solvent, *n*-hexane or *n*-pentane and $\approx 10\ \mathrm{cm}^3$ of these mixtures were used to carryout reactions. Then, $\approx 0.10\ \mathrm{cm}^3$ of a solution containing the enzyme [0.010 g of ketoreductase dissolved in $0.10\ \mathrm{cm}^3$ of phosphate buffer {K_2HPO_4 ($c = 0.10\ \mathrm{mol \cdot dm^{-3}}$), adjusted to pH 7.3 with H_3PO_4} containing NAD(red) ($c = 1.0 \cdot 10^{-3}\ \mathrm{mol \cdot dm^{-3}}$)] was added to each reaction mixture. These reaction mixtures were then placed in a constant temperature bath (±0.01 K) and shaken laterally at $\approx 30\ \mathrm{shakes \cdot min^{-1}}$. The solutions were periodically analyzed by g.c. to determine the extent of reaction. A reaction was considered to be at equilibrium when the ratios of the g.c. peak of products/reactants were essentially identical for the forward and the reverse reaction mixtures. All the reactions that were studied required the addition of freshly prepared enzyme solution in order to achieve equilibrium within 7–15 days. One to three such enzyme additions (the same amount of enzyme as was used initially) were required.

The method of analysis of the reactants and products in the equilibrated reaction mixtures in *n*-hexane and *n*-pentane was similar to the method described previously [21,22]. For quantitative analysis $\approx 4\ \mathrm{cm}^3$ of reaction mixture and $\approx 0.10\ \mathrm{cm}^3$ of internal standard solution (1-hexanol) were gravimetrically added to a vial, and tightly capped. Approximately, $6 \cdot 10^{-4}\ \mathrm{cm}^3$ 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 $(\mathrm{kg \cdot soln})^{-1}$\} of each of the substances involved in the reactions were determined from their respective chromatographic peak areas, the response factor ratios, the concentration of the internal standard solution, and the chromatographic peak area of the internal standard.

3. Results and discussion

3.1. Thermodynamic formalism

The respective equilibrium constants for reactions (1) to (6) are

$$K = c(\text{1-phenyl ethanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{acetophenone(soln)}) \cdot c(\text{2-propanol(soln)})\}, \quad (10)$$

$$K = c(\text{1-phenyl-1-propanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{propiophenone(soln)}) \cdot c(\text{2-propanol(soln)})\}, \quad (11)$$

$$K = c(\text{1-phenyl-1-butanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{butyrophenone(soln)}) \cdot c(\text{2-propanol(soln)})\}, \quad (12)$$

$$K = c(\text{1-phenyl-1-pentanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{1-phenyl-1-pentanone(soln)}) \cdot c(\text{2-propanol(soln)})\}, \quad (13)$$

$$K = c(\text{1-phenyl-1-hexanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{1-phenyl-1-hexanone(soln)}) \cdot c(\text{2-propanol(soln)})\}, \quad (14)$$

$$K = c(\text{1-phenyl-1-heptanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{1-phenyl-1-heptanone(soln)}) \cdot c(\text{2-propanol(soln)})\}. \quad (15)$$

Here, c is the concentration of the indicated substance in the organic solvent expressed as mol $(\mathrm{kg \cdot sln})^{-1}$. Since these reactions are symmetrical with respect to reactant and products, the equilibrium constants of these reactions are dimensionless quantities and are independent of the units chosen to express the concentrations. 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.

If we consider reactions (1) to (6) in terms of the formation of a specific chiral product, the generic reaction for the (*R*)-(+)-1-phenyl-1-alkanol isomer, is

$$\text{1-phenyl-1-alkanone(soln)} + \text{2-propanol(soln)} = (R)\text{-}(+)\text{-1-phenyl-1-alkanol(soln)} + \text{acetone(soln)}. \quad (16)$$

The equilibrium constant for reaction (16) is

$$K = c\{(R)\text{-}(+)\text{-1-phenyl-1-alkanol(soln)}\} \cdot c(\text{acetone(soln)}) / \{c(\text{1-phenyl-1-alkanone(soln)}) \cdot c(\text{2-propanol(soln)})\}. \quad (17)$$

The equilibrium constant for the generalized reaction (9) involving the racemic mixture {equal amounts of the (*R*) and (*S*) isomers of 1-phenyl-1-alkanol} is

$$K = c(\text{1-phenyl-1-alkanol(soln)}) \cdot c(\text{acetone(soln)}) / \{c(\text{1-phenyl-1-alkanone(soln)}) \cdot c(\text{2-propanol(soln)})\}. \quad (18)$$

Here,

$$c(\text{1-phenyl-1-alkanol(soln)}) = c\{(R)\text{-}(+)\text{-1-phenyl-1-alkanol(soln)}\} + c\{(S)\text{-}(-)\text{-1-phenyl-1-alkanol(soln)}\}. \quad (19)$$

But the g.c. analysis of the reaction mixture has shown that the products are a racemic mixture {equal amounts of (*R*) and (*S*) isomers}. Thus,

$$c(\text{1-phenyl-1-alkanol}) = 2 \cdot c\{(R)\text{-}(+)\text{-1-phenyl-1-alkanol}\} = 2 \cdot c\{(S)\text{-}(-)\text{-1-phenyl-1-alkanol}\}, \quad (20)$$

This leads to

$$K(18) = 2 \cdot K(17). \quad (21)$$

Henceforth, we will be dealing with equilibrium constants for reactions (1) to (6) as defined by equations 10 to 15, where the total amount of the 1-phenyl-1-alkanol is considered. The equilibrium constants for specific chiral products can be calculated by using equation (21).

TABLE 2
Results of equilibrium measurements of the ketoreductase-catalyzed reduction of 1-phenyl-1-alkanone to the corresponding 1-phenyl-1-alkanol carried out in *n*-pentane and *n*-hexane {reactions (1) to (6)}

Solvent	Direction	*T*/K	*c*(phenylalkanone)	*c*(2-propanol)	*c*(phenylalkanol)	c(acetone)	K	⟨*K*⟩
		K	mol · (kg soln)$^{-1}$	mol · (kg soln)$^{-1}$	mol · (kg soln)$^{-1}$	mol · (kg soln)$^{-1}$		
1-Phenyl-1-ethanone(soln) + 2-propanol(soln) = 1-phenyl-1-ethanol(soln) + acetone(soln) (1)								
n-Hexane	Forward	288.36	0.06074	0.00730	0.00618	0.01456	0.2029 ± 0.0025	0.2033 ± 0.0048
	Reverse	288.36	0.06127	0.00669	0.00491	0.01700	0.2036 ± 0.0024	
	Forward	292.83	0.05860	0.00701	0.00422	0.02069	0.2125 ± 0.0016	0.2132 ± 0.0054
	Reverse	292.83	0.06214	0.00615	0.00387	0.02111	0.2138 ± 0.0009	
	Forward	297.93	0.06170	0.00900	0.00525	0.02275	0.2151 ± 0.0018	0.2171 ± 0.0050
	Reverse	297.93	0.04967	0.00767	0.00492	0.01696	0.2190 ± 0.0008	
	Forward	303.04	0.06252	0.00866	0.00490	0.02467	0.2233 ± 0.0020	0.2227 ± 0.0052
	Reverse	303.04	0.06253	0.00863	0.00522	0.02296	0.2221 ± 0.0019	
	Forward	308.05	0.05895	0.01032	0.00542	0.02601	0.2317 ± 0.0012	0.2317 ± 0.0056
	Reverse	308.05	0.06232	0.00935	0.00473	0.02853	0.2316 ± 0.0019	
n-Pentane	Forward	298.15	0.01278	0.01337	0.00296	0.01207	0.2091 ± 0.0022	0.2089 ± 0.0053
	Reverse	298.15	0.01168	0.00668	0.00185	0.00880	0.2087 ± 0.0019	
1-Phenyl-1-propanone (soln) + 2-propanol(soln) = 1-phenyl-1-propanol(soln) + acetone(soln) (2)								
n-Hexane	Forward	298.15	0.00869	0.00159	0.000509	0.00919	0.3385 ± 0.0046	0.3401 ± 0.0086
	Reverse	298.15	0.01073	0.00335	0.000648	0.01895	0.3416 ± 0.0068	
n-Pentane	Forward	298.15	0.01754	0.00231	0.000694	0.02115	0.3623 ± 0.0028	0.3626 ± 0.0094
	Reverse	298.15	0.01710	0.00315	0.001262	0.01549	0.3629 ± 0.0045	
1-Phenyl-1-butanone(soln) + 2-propanol(soln) = 1-phenyl-1-butanol(soln) + acetone(soln) (3)								
n-Hexane	Forward	298.15	0.00949	0.00164	0.000485	0.00823	0.2565 ± 0.0063	0.2562 ± 0.0067
	Reverse	298.15	0.01032	0.00318	0.000492	0.01706	0.2558 ± 0.0076	
n-Pentane	Forward	298.15	0.01262	0.00248	0.000400	0.02059	0.2632 ± 0.0012	0.2648 ± 0.0062
	Reverse	298.15	0.01221	0.00141	0.000327	0.01402	0.2663 ± 0.0087	
1-Phenyl-1-pentanone(soln) + 2-propanol(soln) = 1-phenyl-1-pentanol(soln) + acetone(soln) (4)								
n-Hexane	Forward	298.15	0.01114	0.00462	0.00113	0.02165	0.4753 ± 0.0024	0.4797 ± 0.0119
	Reverse	298.15	0.01105	0.00474	0.00110	0.02305	0.4841 ± 0.0048	
n-Pentane	Forward	298.15	0.01355	0.00214	0.000776	0.01810	0.4844 ± 0.0064	0.4870 ± 0.0123
	Reverse	298.15	0.02034	0.002844	0.00145	0.01953	0.4895 ± 0.0048	
1-Phenyl-1-hexanone(soln) + 2-propanol(soln) = 1-phenyl-1-hexanol(soln) + acetone(soln) (5)								
n-Hexane	Forward	298.15	0.02735	0.02695	0.01499	0.01514	0.3079 ± 0.0064	0.3167 ± 0.061
	Reverse	298.15	0.03293	0.03191	0.01325	0.02581	0.3255 ± 0.0037	
n-Pentane	Forward	298.15	0.02830	0.02421	0.01423	0.01411	0.2931 ± 0.0048	0.2976 ± 0.0049
	Reverse	298.15	0.02932	0.03151	0.01538	0.01815	0.3021 ± 0.0031	
1-Phenyl-1-heptanone(soln) + 2-propanol(soln) = 1-phenyl-1-heptanol(soln) + acetone(soln) (6)								
n-Hexane	Forward	298.15	0.01655	0.03293	0.01826	0.01695	0.5679 ± 0.0053	0.5697 ± 0.0167
	Reverse	298.15	0.02832	0.02845	0.01448	0.03180	0.5715 ± 0.0056	
n-Pentane	Forward	298.15	0.01866	0.01129	0.01132	0.01035	0.5561 ± 0.0074	0.5667 ± 0.0169
	Reverse	298.15	0.01732	0.01730	0.01540	0.01123	0.5772 ± 0.0071	

The 1-phenyl-1-alkanones are: 1-phenyl-1-ethanone, 1-phenyl-1-propanone, 1-phenyl-1-butanone, 1-phenyl-1-pentanone, 1-phenyl-1-hexanone and 1-phenyl-1-heptanone. The concentrations of the substrates involved in the reaction {1-phenyl-1-alkanone (soln) + 2-propanol(soln) = 1-phenyl-1-alkanol (soln) + acetone(soln)} are given in columns 4 to 7; each reported concentration is an average of five measurements. The values of the equilibrium constants *K* listed in column 8 are calculated from the concentrations given in columns 4 to 7 and by using equations (10) to (15). The quantity ⟨*K*⟩ is the average of the equilibrium constants which were obtained from the forward and the reverse directions of a reaction. The uncertainties in the values of *K* (column 8) are based on two estimated standard deviations of the mean. The uncertainties given in column 9 are the combined expanded uncertainties in the values of ⟨*K*⟩.

3.2. Results of equilibrium measurements

The equilibrium constants for the ketoreductase-catalyzed reduction of 1-phenyl-1-alkanone to the corresponding 1-phenyl-1-alkanol are given in table 2. This table includes equilibrium constants for reactions (1) to (6) in *n*-hexane and *n*-pentane at 298.15 K and also the equilibrium constants for reaction (1) in *n*-hexane as function of temperature. The equilibrium constant K listed in column 8 was calculated from the "c" of reactants and products, reported in columns 4 to 7 and is an average of five measurements. The uncertainties given in this column 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. The equilibrium constants $\langle K \rangle$ reported in the last column are the averages of the equilibrium constants obtained from the forward and the reverse directions. It is important to note that the values of the equilibrium constants obtained from the forward and the reverse directions of a reaction are generally in agreement within their statistical uncertainties. It is judged that reasonable estimates of the standard uncertainties [25] due to possible systematic errors in the values of the equilibrium constants for these reactions are: $\pm 0.05 \cdot \mathrm{K}$ 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 $\pm 0.01 \cdot \mathrm{K}$ 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 combined standard uncertainties [25]. These combined expanded uncertainties are reported in the last column with the average values of the equilibrium constants $\langle K \rangle$ (see column 9 in table 2).

For reaction (1) we have calculated 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. In this calculation, we have used the Clark and Glew equation [26] with the assumption that the standard molar heat capacity change $\Delta_r C^\circ_{p,m}$ for this reaction is zero. The calculated values for the thermodynamic quantities at $T = 298.15$ K are: $K = 0.2177 \pm 0.0018$; $\Delta_r G^\circ_m = (3.78 \pm 0.02)\ \mathrm{kJ \cdot mol^{-1}}$; $\Delta_r H^\circ_m = (4.53 \pm 0.87)\ \mathrm{kJ \cdot mol^{-1}}$, and $\Delta_r S^\circ_m = (2.5 \pm 2.9)\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$. These uncertainties are equal to two estimated standard deviations of the mean.

The chiral g.c. analysis of the reaction mixture has shown that in every reaction the products were a racemic mixture {equimolar mixture of (*R*)-(+)-1-phenyl-1-alkanol and (*S*)-(−)-1-phenyl-1-alkanol isomers}. Thus, the ketoreductase used in our study for reduction of 1-phenyl-1-alkanones was not stereoselective. However, Zhu *et al.* [16] have recently used NADP(red) dependent ketoreductase which were produced *via* genomic mining and protein engineering. In their study they have coupled the ketoreductase with D-glucose dehydrogenase and D-glucose for regeneration of NADP(red) and have investigated the stereoselective reduction of a series of arylketones.

The present study of 1-phenyl-1-alkanone is similar to our previous studies of 2-alkanone reactions [21]. Comparing the two studies, the phenyl group in the 1-phenyl-1-alkanone reaction replaces the methyl group of the 2-alkanone reactions. In 2-alkanone reactions [21], the values of the equilibrium constants decrease monotonically with increasing value of the number of carbons and approaches a limiting value of ≈0.30 for the number of carbons >8. In figure 2, the equilibrium constants for reactions (1) to (6) carried out in *n*-hexane at $T = 298.15$ K is plotted as a function of the number of carbons N_C in the alkanone side chain in the 1-phenyl-1-alkanones. In 1-phenyl-1-alkanone reactions, the presence of the phenyl group has a pronounced effect on the values of the equilibrium constants. The values of the equilibrium constants of 1-phenyl-1-alkanone with an odd number of carbons in alkyl side chain are higher than the values of the equilibrium constants of the preceding 1-phenyl-1-alkanone having an even number of carbons in the side chain. Here, the values of the equilibrium constants of 1-phenyl-1-alkanone systems follow a zig-zag pattern with increasing carbon number in the alkyl

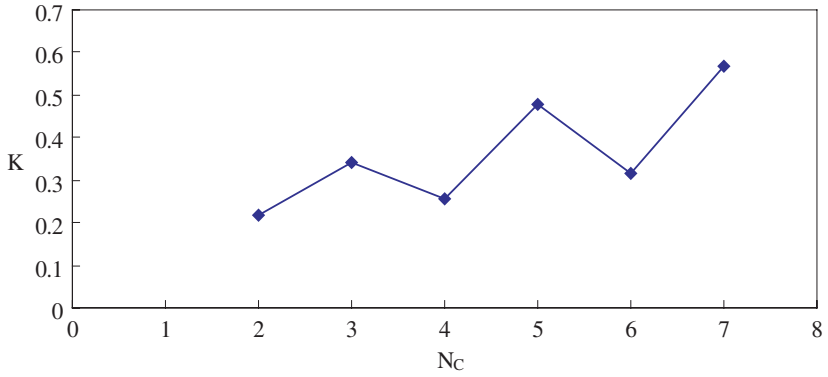

FIGURE 2. The equilibrium constant K at $T = 298.15$ K for the reduction of 1-phenyl-1-alkanones {reactions (1) to (6)} in *n*-hexane plotted against the number of carbons N_C in the alkanone side chain.

side chain. Presently, we are unable to offer an explanations for this behavior of 1-phenyl-1-alkanone reduction.

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