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Enzymatic Enantioseparation of Bicyclo[3.3.1]nonane-2,6-diones

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Summary. Preparative enantioseparation of the (+)- and (−)-enantiomers of bicyclo[3.3.1]nonane-2,6-dione was performed by means of a horse liver alcohol dehydrogenase catalyzed reduction, coupled with the regeneration of the coenzyme *NAD* by dithionite or ethanol.

Keywords. Bicyclo[3.3.1]nonanes; Enantioseparation; Enzymatic reduction; Alcohol dehydrogenase; Stereochemistry.

Enzymatische Enantiomerentrennung von Bicyclo[3.3.1]nonan-2,6-dionen

Zusammenfassung. Eine präparative enzymatische Trennung der (+)- und (−)-Enantiomeren von Bicyclo[3.3.1]nonan-2,6-dion unter Verwendung von Pferdeleber-Alkoholdehydrogenase, verbunden mit *In-situ*-Regenerierung des Koenzymes *NAD* mit Dithionit und Ethanol, wurde durchgeführt.

Introduction

Bicyclo[3.3.1]nonanes have attracted considerable interest in recent years. Carbonyl derivatives of bicyclo[3.3.1]nonane are considered as precursors in organic synthesis and useful models in conformational analysis. The introduction of a carbonyl group into definite positions of the bicyclo[3.3.1]nonane skeleton induces chirality of the latter. Thus, the existence of enantiomers of carbonyl derivatives of bicyclo[3.3.1]nonane is possible. Among these compounds, bicyclo[3.3.1]nonane-2,6-dione (**1**) seems to be of great interest. Enantiomerically enriched (+)-**1** was synthesized for the first time by reductive desulfurization of 2-thiaadamantane-4,8-dione, and was obtained in optically active form ($ee = 2\%$) by means of preparative chromatography on acetylcellulose [1]. Later, (+)-**1** was obtained by oxidation of the corresponding enantiomeric diols prepared by chemical resolution [2]. The absolute configuration of (+)-**1** was assigned as (1*S*,5*S*) [1, 2]. On a preparative scale, the resolution of the enantiomers of **1** was performed by stereoselective reduction using bakers yeast [3]. (+)-(1*S*,5*S*)-**1** was

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obtained in high enantiomeric excess (60–93%) as the remaining enantiomer, non-active in yeast-catalyzed reduction, whereas (−)-(1*R*,5*R*)-**1** was prepared with an *ee* of 58% by oxidation of the yeast-catalyzed reaction products [3]. Chromatography of **1** on chiral microcrystalline triacetylcellulose yielded enantiomerically pure (−)- and (+)-diones [4]. Their absolute configurations were assigned as (−)-(1*R*,5*R*)-**1** and (+)-(1*S*,5*S*)-**1** based on circular dichroism spectra [4] and by means of computational methods [5, 6]. Up to now, the preparative synthesis of enantiomeric bicyclo[3.3.1]nonane-2,6-diones still remains an unsolved problem.

Biocatalysis is often considered as one of the most promising synthetic techniques in synthesis and resolution of enantiomers [7]. One of the most prominent biocatalysts in this respect is horse liver alcohol dehydrogenase (HLADH) which is able to catalyze reversible redox reactions between coenzyme *NAD* or *NADH* and a wide variety of alcohols and ketones [8]. The aim of the present work was a preparative resolution of (±)-**1** into its enantiomers using of HLADH-catalyzed redox transformations.

Results and Discussion

Spectrokinetic investigations showed that **1** possessed activity as a substrate in HLADH-catalyzed reduction by coenzyme *NAD*H. The reaction rate (v) in these experiments was recorded monitoring the disappearance of the *NAD*H absorbance band in the near UV region and plotted in *Lineweaver-Burk* coordinates ($1/v$ *vs.* $1/c$) as a function of the concentration of **1** (Fig. 1). A linear dependence thus obtained could be treated in a usual way following *Michaelis-Menten* kinetics, yielding a *Michaelis* constant value of $K_M = 57\,mM$.

Since the expensive coenzyme *NAD*H is consumed in the HLADH-catalyzed reduction of **1** in equimolar quantity, the preparative synthesis is in need of a coupled continuous regeneration of the coenzyme. In the present work,

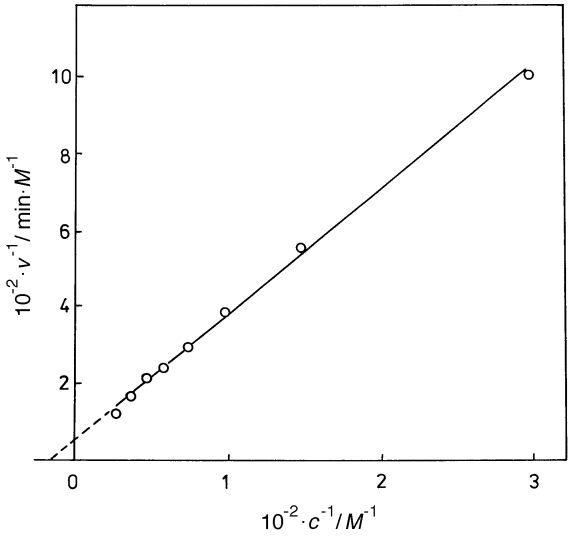


Fig. 1. *Lineweaver-Burk* plot for HLADH-catalyzed reduction of **1**; for details, see Experimental

regeneration of *NAD*H was performed following two different ways, either by addition of dithionite (method A) or by using an excess of ethanol (method B). In the latter case, the oxidized form of the coenzyme (NAD^+) is regenerated to its reduced form (*NAD*H) in the enzymatic reaction catalyzed by HLADH according to Eqs. (1) and (2).

$$\mathbf{1} + NADH + H^+ \rightarrow \text{Products} + NAD^+ \quad (1)$$

$$NAD^+ + C_2H_5OH \rightarrow NADH + H^+ + CH_3CHO \quad (2)$$

Since both reactions are catalyzed by the same enzyme, a coupled substrate recycling results in the reduction of **1** by ethanol (Eq. (3)).

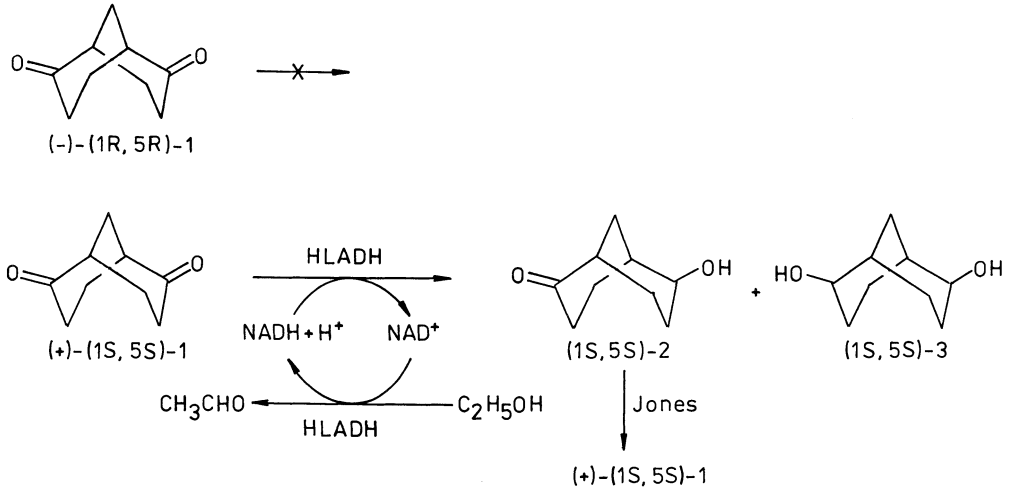
$$\mathbf{1} + C_2H_5OH \rightarrow \text{Products} + CH_3CHO \quad (3)$$

In case of a molar excess of the reducing agent (*i.e.* dithionite or ethanol) relative to **1**, the equilibrium of the coupled process (3) should be strongly shifted towards the products of enzymatic reduction of **1**. In the present work, this was achieved by the use of a 16-fold molar excess of dithionite (method A) or a 42-fold molar excess of ethanol (method B). The performance of a coupled process with the regeneration of coenzyme allows to use much lower amounts of *NAD* than demanded by the stoichiometry of Eq. (1). In the present work, a molar ratio of *NAD* to **1** as low as 10:1 or 30:1 was used, permitting to perform a preparative synthesis in the range of *ca*. 1 g of **1**.

It was established by thin layer chromatography that, even at long incubation times, the amount of converted **1** did not exceed 30 to 50%. After the conversion is completed, 6-hydroxy-bicyclo[3.3.1]nonane-2-one (**2**) was found as the main reaction product next to a small amount of bicyclo[3.3.1]nonane-2,6-diol (**3**). The amount of **3**, as compared to that of **2**, was found to be higher upon using method B. Also, a higher degree of conversion of **1** to **2** and **3** was observed in this case. It therefore follows that HLADH-catalyzed regeneration of *NAD* by means of ethanol is more efficient than the dithionite-assisted process.

After the enzymatic conversion was completed, unreacted **1** could be easily extracted from the reaction mixture by means of organic solvents and purified by column liquid chromatography. Unreacted **1** obtained in this way displayed optical activity which increased with increasing conversion degree of **1** up to 50%. Optically active (−)-**1** obtained in this way showed lower activity in an enzymatic reaction when compared to racemic **1**. (−)-**1** obtained by method A showed an $[\alpha]_D$ value of $-66°$ and a relative activity in the HLADH-catalyzed reaction of 59% as compared to racemic **1**, whereas the corresponding values for (−)-**1** obtained by method B were $-149°$ and 37%.

It could be concluded from this result that only (+)-**1** undergoes an enzymatic reduction in the HLADH-catalyzed reaction, whereas (−)-**1** remains unconverted. The extrapolation of $[\alpha]_D$ and the relative activity in enzymatic reaction to zero for the latter, provided for the samples of (−)-**1** and obtained by varying of reaction conditions, yield an $[\alpha]_D$ value of $-197°$ for enantiomerically pure (−)-**1**. The value obtained is closely related to that reported for (+)-**1** ($[\alpha]_D = +191°$ [2]). The oxidation of the main enzyme reaction product **2** by dichromate following the well-known *Jones* procedure yielded (+)-**1** with $[\alpha]_D = +119°$. The interconversions of the bicyclic compounds studied are shown in Scheme 1.



Scheme 1

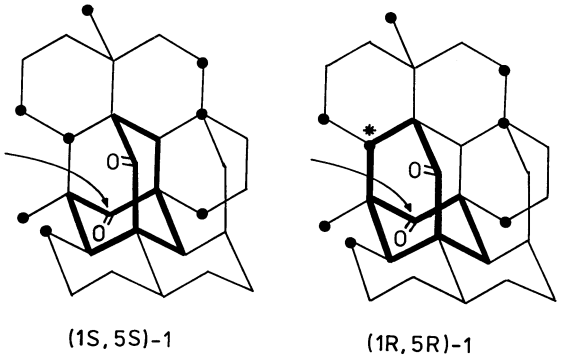


Fig. 2. Projections of (1*S*,5*S*)- and (1*R*-5*R*)-enantiomers of **1** into a 'diamond lattice' model of the active center of HLADH; the arrows indicate the site of enzyme-catalyzed reduction of the keto group; undesirable positions are marked by full circles

It was of great interest to assign the absolute configuration of the enantiomers obtained. Following a known assignment [1, 2] (+)-**1** can be defined as (+)-(1*S*,5*S*)-**1**. Thus, the non-active in enzymatic reaction enantiomer can be assigned as (−)-(1*R*,5*R*)-**1**. The same conclusion can be drawn also following an independent way, based on the 'diamond lattice' model of the active site of HLADH [10]. Figure 2 shows the projections of (1*S*,5*S*)-and (1*R*,5*R*)-enantiomers into a 'diamond lattice' of HLADH. It can be seen that the (1*S*,5*S*)-enantiomer can be placed into the 'diamond lattice' in such a way that no carbon atom of its skeleton occupies an undesirable position. In contrast, placing (1*R*,5*R*)-**1** into this model requires the occupation of one of the undesirable positions (as indicated by an asterisk in Fig. 2). Thus, from both enantiomers of **1**, only the (1*S*,5*S*)-enantiomer can be bound into an active center of HLADH enabling an enzyme-catalyzed reaction to proceed.

Experimental

Horse liver alcohol dehydrogenase (activity: 2 units/mg) in the form of a suspension in ammonium sulfate solution containing 19 mg/cm^3 of enzyme and oxidized and reduced forms of the coenzyme nicotineamide adeninedinucleotide (*NAD* and *NAD*H) were obtained from Reanal (Hungary) and used as received. (±)-Bicyclo[3.3.1]nonan-2,6-dione (**1**) was synthesized according to a known procedure [9].

The kinetics of the enzyme-catalyzed reduction of **1** was studied using spectrophotometer Specord UV-Vis (Germany) equipped with a thermostatted cuvette holder. All kinetic experiments were performed at 25°C. For kinetic investigations, 1 cm^3 of a solution of **1** in 0.1 *M* phosphate buffer ($pH = 7.5$) was mixed with 0.112 cm^3 of 5 m*M* *NAD*H solution in the same buffer. The total concentration of **1** in the kinetic experiments was varied within the range of 4 to 40 m*M*. The reaction was started by addition of 0.087 cm^3 of HLADH solution containing 0.033 mg of the enzyme. The rate of enzymatic oxidation of *NAD*H was monitored at 370 nm. The optical activity of the solutions of **1** in dioxane ($c = 0.2$–0.3 g in 100 cm^3 of the solvent) was recorded in a 10 cm path length cuvette by means of a Perkin-Elmer model 141 polarimeter.

Preparative synthesis of optical active enantiomers of **1** was performed according to two different methods as follows:

Method A. 456 mg (3 mmol) of **1** was dissolved in 250 cm^3 of 0.1 *M* phosphate buffer ($pH = 7.5$) together with 210 mg (0.3 mmol) of *NAD* and 8.7 g (50 mmol) of sodium dithionite. Then, 2 cm^3 of *HLADH* suspension containing 38 mg of the enzyme were added. The reaction mixture was kept for 24 h at 30°C, then evaporated *in vacuo* to a final volume of *ca*. 50 cm^3, and extracted exhaustively with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated *in vacuo*. The rest was dissolved in a minimum volume of CH_2Cl_2 and fractionated by liquid chromatography (solvent: CH_2Cl_2) on an alumina (2nd grade of activity) column. The fraction containing **1** ($R_f = 0.69$) was dried, evaporated, and handled in the usual manner. Yield: 170 mg; $[\alpha]_D = -66°$.

Method B. A similar procedure was used, except that a lower amount of *NAD* (70 mg; 0.1 mmol) and 7.2 cm^3 (125 mmol) of ethanol instead of sodium dithionite were added to the reaction mixture. Yield 182 mg; $[\alpha]_D = -149°$.

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