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Original Scientific Paper

A Study of Enantioselective Reduction of *para*-Substituted 2-Methyl-cinnamaldehydes by Baker's Yeast*

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Reduction of a series of *para*-substituted 2-methyl-cinnamaldehydes (**1–7**) at 20 °C and at pH = 2–3 afforded *S*-enantiomers of saturated alcohols **8–14** in 20–80% yield and 75 – ≥ 99% enantiomeric excess (e.e.); at 30 °C, lower yields and e.e.s were obtained. Relative rates of the formation of allylic alcohols **15–21**, catalyzed by alcohol dehydrogenase (ADH), correlate with the Hammett σ^+ values of *para*-substituents, revealing that a more efficient delocalization of the positive charge on carbonylic carbon slow down the reduction rates, whereas no correlation of the electronic properties of the substituents with the rate of C=C double bond reduction, catalyzed by enoate reductase, is observed. On reduction of **3** by dried yeast in ^2H_2O, α,β-carbon atoms in **10a** bear ^2H atoms, in accordance with the previously reported hydrogenation of selectively ^2H-labeled cinnamic aldehyde and cinnamic alcohol. The accumulated data indicate that the mechanism of the enone C=C bond reduction that comprises nucleophilic attack of the hydride ion species on the β-carbon in the first step, followed by enantioselective protonation on the α-carbon atom.

INTRODUCTION

In his already classical papers,[1] Prelog has proposed a model to explain the enantioselectivity of hydrogenation of prochiral ketones by NAD(P)H dependent alcohol dehydrogenase (ADH) from the baker's yeast (b.y., *Saccharomyces cerevisiae*), Figure 1.

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* Dedicated to Professor Vladimir Prelog on the occasion of his 90th birthday.

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Figure 1. Facsimile of Prelog's original proposal of the model for enantioselective reduction of ketones by *Sacharomyces cerevisiae.*[1b]

The paper from 1964[1b] has become one of the most cited works in the literature on bioorganic chemistry, and the model has been proven on a plethora of prochiral ketones.[2–4] Reminiscences of the events related to the birth of this model can be found in this Issue.[5]

The cells of b.y. represent a living natural pool of hundreds of enzymes that are able to display various transformations of organic molecules.[6] Their capability to reduce the activated (conjugated) carbon-carbon double bond is particularly attractive for preparation of important organic target molecules.[7,8] This property has recently been properly named »new synthetic capacity of baker's yeast«.[9] In this reduction, the action of enoate reductase is regularly invoked.[10,11] Reduction of carbonyl group and conjugated C=C bond by enzymes and microbial cells has found its place in recent monograph on organic synthesis, among other chemical catalytic and noncatalytic methods of hydrogenation,[12a] and also in a compendium of biocatalytic synthetic methods.[12b]

A general explanation for the stereochemical course of microbial reduction of enones was first proposed by Kergomard *et al*,[13] who studied reduc-

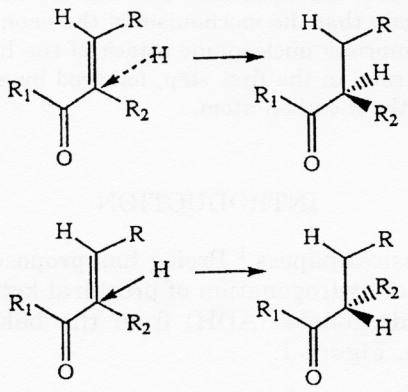


Figure 2. Model of Kergomard *et al.* proposed for enantioselective reduction of α,β-unsaturated ketones by *Beauveria sulfurescens.*[10]

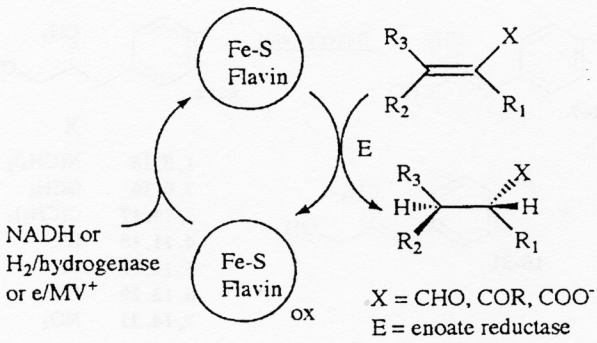
tion of α,β-unsaturated ketones by *Beauveria sulfurescens*. They have classified two double bond substituents α to the carbonyl analogously to Prelog's model,[1] Figure 2, and postulated that the »attack of hydrogen on this carbon occurs according to the same stereochemistry as on the carbonyl carbon, *i.e.* from behind if the larger substituent is on the left and from the front if the larger substituent is on the right«.[13]

Absolute stereochemistry of the b.y. catalyzed hydrogen addition on the C=C bond in enones is also discussed by Servi in his recent review,[6] and the rationale of the observed configurations at the newly formed chiral centre is based on the model for type **I** and type **II** substrates.

G = CH_2OH, CHO, $CH_2(OR^1)_2$

Although this rule correctly accounts for the stereochemistry of reduction of various enones, it formally envisages first attack of hydrogen on the α-carbon, well known to bear a partial negative charge in such 4π unsaturated systems.[14] This hydrogen should therefore be delivered from the enzyme as a proton or as a proton-like species that bears positive charge.[14] The same group has reported[15] the results of an elegant study revealing *trans*-addition of hydrogen across the double bond, *pro-R* hydrogen atom being delivered to the α-carbon atom.

As to the mechanistic course of the microbial reduction of enones, Simon has proposed the mechanism of reduction with 2-enoate reductase (E.C. 1.3.1.31) from *Clostridium kluyveri*, as outlined in Scheme 1.[16,17]

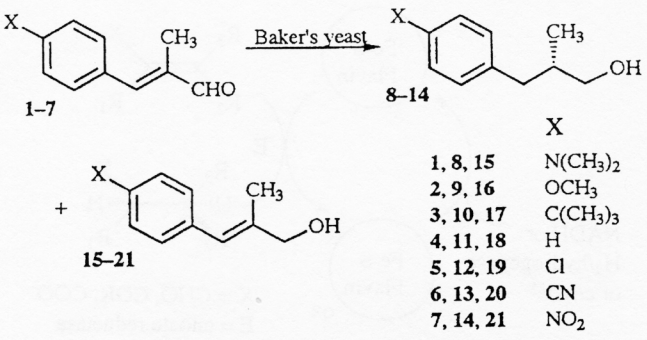


Scheme 1. Reduction with 2-enoate reductase proposed by Simon *et al.*[16,17]

In practice, whole cells are usually used for the reduction of enones, presumably due to the instability of the free enzyme, which contains an iron-sulfur-flavin cluster at the active site. Prompted by certain ambiguity, if hydride or proton is added first during *trans*-addition, and by our recent successful application of b.y. in the chemoenzymatic synthesis of *S*-enantiomers of some systemic fungicides, wherein enantioselective step comprises reduction of an 2-methyl-cinnamaldehyde derivative,[18] we have undertaken a more detailed study of the b.y. catalyzed reduction of a series of *para*-substituted 2-methyl-cinnamaldehydes. Though aware of the ill-defined biocatalytic system, we herewith report the results of the relative rates and enantioselectivity of reduction in this series of compounds, expecting that they will contribute to a better understanding of this process and broaden the application of b.y. in the enantioselective reduction of activated C=C bonds.

RESULTS AND DISCUSSION

A series of *para*-substituted derivatives of 2-methyl-cinnamaldehyde, in Scheme 2, have been prepared by aldol condensation of the corresponding *para*-substituted benzaldehydes with propionaldehyde. The reactivity of the aldehydes was strongly dependent on the *para*-substituent; strong electron-withdrawing substituents promoted in *ca.* 3 h at room temperature, quantitative conversion to 2-methyl-cinnamaldehyde derivatives, whereas electron-donating substituents slowed the reaction; from *para*-dimethylamino benzaldehyde, compound **1** was obtained in 39% yield after 40 h under reflux and *ca.* 45% of the starting aldehyde remained unreacted, as determined by GLC. The effect of electron-donating *para*-substituents revealed that delocalization of the partial positive charge on the aldehydic carbon



Scheme 2. Reduction of **1–7**.

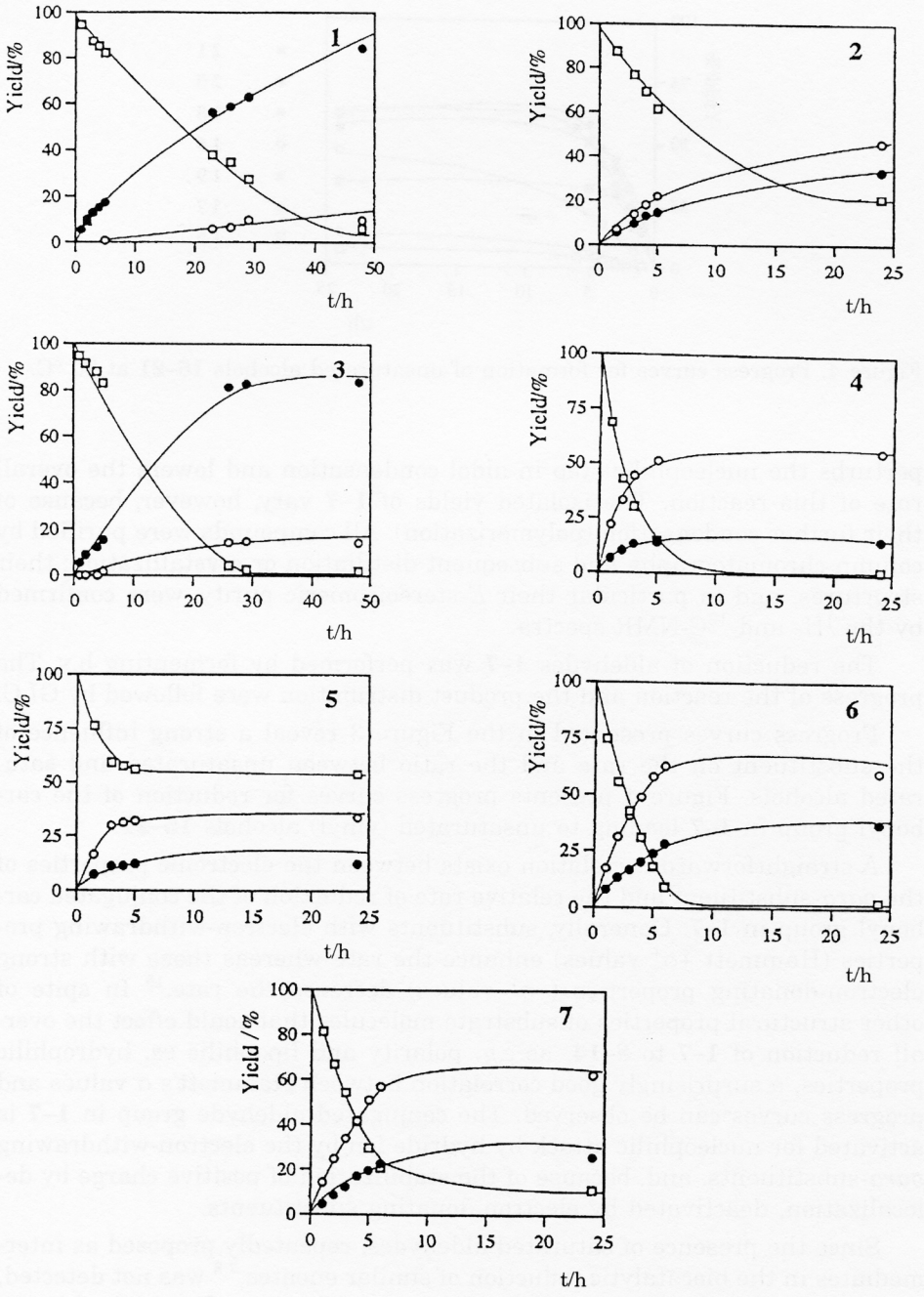


Figure 3. Progress curves for reduction of **1–7** with fresh b. y. at 20 °C; □–□–□ starting aldehyde, ●–●–● saturated alcohol, o-o-o unsaturated alcohol.

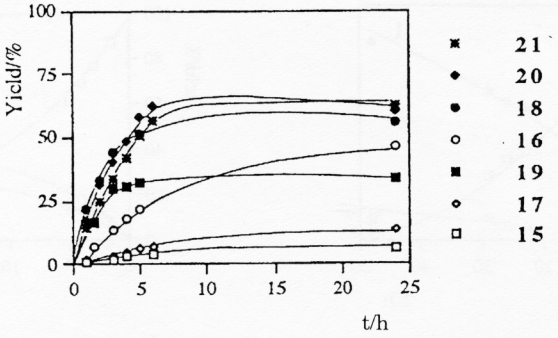


Figure 4. Progress curves for formation of unsaturated alcohols **15–21** at 20 °C.

perturbs the nucleophilic step in aldol condensation and lowers the overall rate of this reaction. The isolated yields of **1–7** vary, however, because of their further condensation (polymerization). All compounds were purified by column chromatography and subsequent distillation or crystallization; their structures, and in particular their *E*-stereoisomeric purity, were confirmed by the ^{1}H- and ^{13}C-NMR spectra.

The reduction of aldehydes **1–7** was performed by fermenting b.y. The progress of the reaction and the product distribution were followed by GLC.

Progress curves presented in the Figure 3 reveal a strong influence of the substituent on the rate and the ratio between unsaturated and saturated alcohols. Figure 4 presents progress curves for reduction of the carbonyl group in **1–7** leading to unsaturated (vinyl) alcohols **15–21**.

A straightforward correlation exists between the electronic properties of the *para*-substituent and the relative rate of reduction of the conjugated carbonyl group in **1–7**. Generally, substituents with electron-withdrawing properties (Hammett $+\sigma^{+}$ values) enhance the rate whereas those with strong electron-donating properties ($-\sigma^{+}$ values) decrease the rate.[19] In spite of other structural properties of substrate molecules that could effect the overall reduction of **1–7** to **8–14**, as *e.g.* polarity and lipophilic *vs.* hydrophilic properties, a surprisingly good correlation between Hammett's σ values and progress curves can be observed. The conjugated aldehyde group in **1–7** is activated for nucleophilic attack by hydride ion by the electron-withdrawing *para*-substituents, and, because of the stabilization of positive charge by delocalization, deactivated by electron-donating substituents.

Since the presence of saturated aldehydes, repeatedly proposed as intermediates in the biocatalytic reduction of similar enoates,[7,8] was not detected, we assumed their very low steady-state concentration. To check this assumption, racemic saturated aldehydes **22**, **23** were prepared and reduced to **10** and **13**, Figure 5.

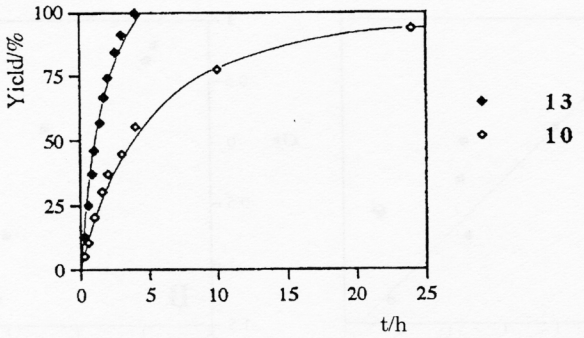


Figure 5. Progress curves for reduction of **22** and **23** to **3** and **6** resp., with fresh b.y. at 20 °C.

Comparing the rates presented in Figure 5 with those for reduction of **3** and **6** into the same saturated alcohols, Figure 3, one can observe a much faster reduction of **22** and **23**. The relatively faster reduction of **22**, as compared to **23**, should be contributed to specific properties, higher hydrophilic or lower steric requirements, of the former. Conversion of both enantiomers of **22** and **23** into alcohols **10** and **13** could be a consequence of either a low degree of kinetic resolution or, a relatively fast racemization *via* keto-enol tautomery. This stereochemical aspect will be addressed in our envisaged study with isolated ADH.

The accumulated evidence suggests that the rate determining step in the reduction of **1–7** to saturated alcohols **8–14** is the hydrogenation of the conjugated C=C bond. No correlation was found between the σ^+ values and the rates of formation of **8–14**.

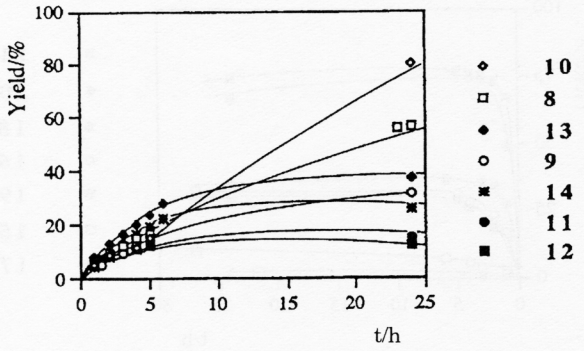


Figure 6. Progress curves for formation of saturated alcohols **8–14** at 20 °C.

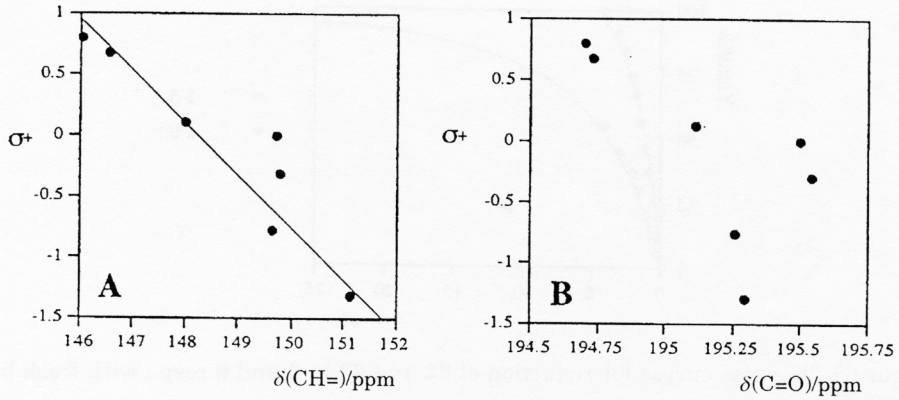


Figure 7. Correlation between σ^+ values and ^{13}C-NMR shifts for β-CH= (**A**) and C=O carbons (**B**).

The electronic effect of substituents is reflected in the ^{13}C shifts of the carbonyl carbon and β-carbon atoms of the C=C bond in **1–7**, Figure 7. $\Delta\delta$-Range for β-carbon atoms is *ca.* 5 ppm, and good correlation between σ^+ and ^{13}C-shift (δ) is observed, Figure 7A. Carbonyl carbon atoms, however, are shifted in a much narrower range, $\Delta\delta \approx 1$ ppm, exhibiting a poor correlation with σ^+ values, Figure 7B.

We have already noticed[18] that the highest conversion and enantioselectivity in the reduction of **3** were obtained at 20 °C; a significant drop of the rate was observed at 30 °C and, therefore, we repeated the reduction of **1–7** at 30 °C. Again, a correlation between σ^+ values and the hydrogenation rate of the aldehyde group was determined, Figure 8, whereas no correlation could be observed for hydrogenation of the C=C bond, Figure 9.

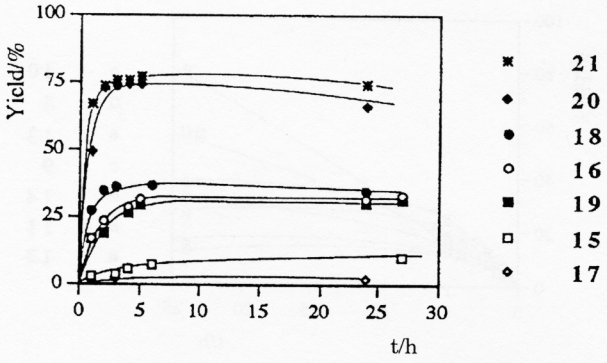


Figure 8. Progress curves for formation of unsaturated alcohols **15–21** at 30 °C.

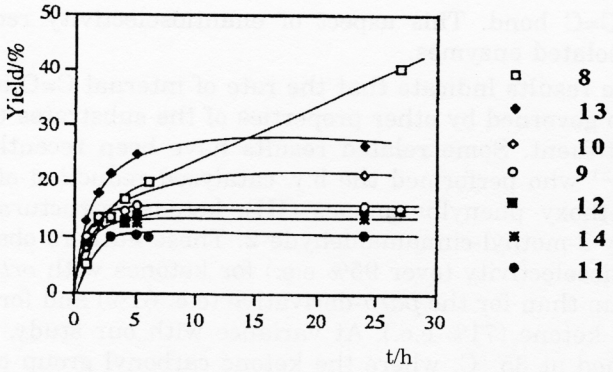


Figure 9. Progress curves for formation of saturated alcohols **8–14** at 30 °C.

In Table I, the yields and e.e.s for the reduction of **1–7** at 20 and 30 °C are collected. Generally, at higher temperature lower yields of **8–14** and higher ratios of **15–21** were recorded, which could be ascribed to the known deactivation of enaoate reductase by oxygen at elevated temperature.[20] Notably higher yields of chiral, saturated alcohols represent the practical benefit of working at lower temperature, as demonstrated in developing a workable procedure for the preparation of systemic fungicides in the enantiomerically pure form.[18]

Another aspect of enantioselectivity deserves to be commented; enantiomeric purity of the products obtained at 20 °C was regularly higher than when the reduction was performed at 30 °C. Besides, a larger drop of e.e. was noticed for derivatives with $+\sigma^+$ substituents than for those with $-\sigma^+$ substituents. This could be explained by either a faster enolization of the intermediary, saturated aldehydes at higher temperature, or by diminished enantioselectivity of the proton-transfer step in hydrogena-

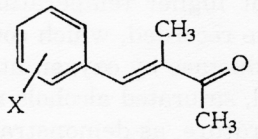
TABLE I

GLC yields and optical purities of chiral alcohols **8–14** at 20 and 30 °C.

Comp.	X	t/h	Yield/% 30 °C	e.e %	t/h	Yield/% 20 °C	e.e %	$[\alpha]_D$ (CH_2Cl_2)
8	$N(CH_3)_2$	27	40.2	92.5	48	84.4	93.6	–6.1
9	OCH_3	27	15.3	62.0	24	33.1	78.3	–4.6
10	$C(CH_3)_3$	24	21.2	81.7	28	83.8	99.0	–6.6
11	H	24	10.3	63.5	24	15.5	75.0	–113.9
12	Cl	27	15.1	50.0	24	12.5	77.9	–22.0
13	CN	24	26.7	66.9	24	37.2	91.8	–15.4
14	NO_2	24	13.5	18.0	24	26.0	88.0	–34.4

tion of the C=C bond. This aspect of enantioselectivity requires further study with isolated enzymes.

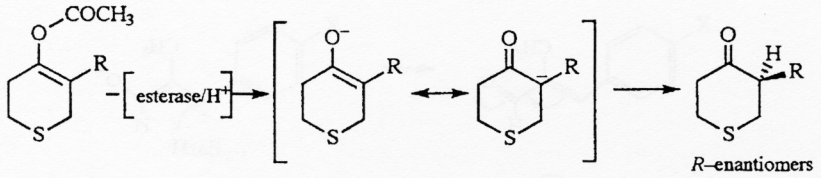
The above results indicate that the rate of internal C=C bond reduction in **1–7** is also governed by other properties of the substrates than the effect of *para*-substituent. Some related results have been recently reported by Kawai *et al.*,[21] who performed the b.y. catalyzed reduction of *ortho*-, *meta*- and *para*-methoxy phenylbutanones (**III**), ketones structurally similar to *para*-methoxy-2-methyl-cinnamaldehyde **2**. These authors observed a much higher enantioselectivity (over 95% e.e.) for ketones with *ortho*- and *meta*-methoxy group than for the *para*-derivative (e.e. 61%) and for the unsubstituted parent ketone (71% e.e.). At variance with our study, all reductions were performed at 35 °C, where the ketone carbonyl group proved unreactive, which resulted in preferential formation of chiral, saturated ketones.[21] This result poignantly reflects the unusual steric demands of enzymatic reactions in comparison with those of more common laboratory reagents.



III X = 2-, 3-, 4-OCH_3

As mentioned in the Introduction, the mechanism of reduction by enoate reductase is repeatedly discussed,[6,13,15] though some stereochemical aspects have not been completely clarified. The general model proposes an »attack of hydrogen« without specifying its anionic (hydride-like) or cationic (proton-like) character. To the best of our knowledge, Fuganti and Servi were the first to propose the formal hydride delivery in the β-position of α,β-unsaturated lactones in order to explain the diastereoselective attack from one of the faces in the lactones.[22,23] It is worth mentioning that chemical hydrogenation of the C=C bond by the first proton attack and then by hydride would require a combination of a strong acid and hydride donor.[24] The biocatalytic equivalent should, therefore, be able to deliver a strongly acidic proton from the active site to the substrate. Such a mechanism seems to be unknown for reductases, and it is thus conceivable that the hydride-ion species is first delivered to the C=C bond. In this case, the β-carbon atom is an obligatory site for nucleophilic attack.

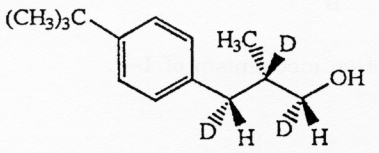
Subsequent protonation at the α-carbon atom in 2-methyl-cinnamaldehides **1–7**, in heterocyclic analogs of 2-methyl-cinnamaldehyde,[8,25] and also in the ketones **III** studied by Kawai *et al.*,[21] comprises a peculiarity; in all these compounds, a chiral centre is created in the protonation step. It should be enantioselective, *i.e.* controlled by the chiral topology of the enzyme active



Scheme 3. Esterase catalyzed enantioselective hydrolysis of ketone-enolates.[26,27]

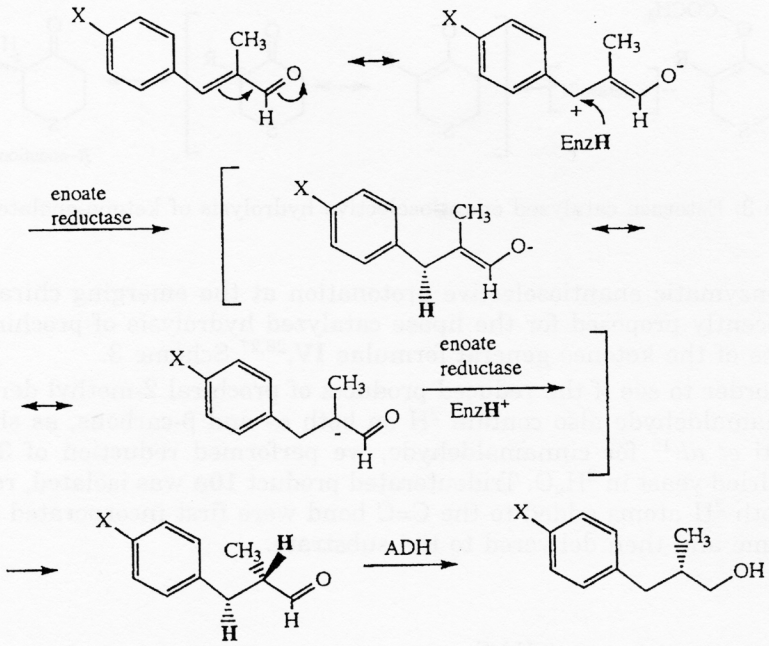
site. Enzymatic enantioselective protonation at the emerging chiral centre was recently proposed for the lipase catalyzed hydrolysis of prochiral enol-acetates of the ketones general formulae **IV**,[26,27] Scheme 3.

In order to see if the reduced products of prochiral 2-methyl derivatives of cinnamaldehyde also contain ^2H on both α- and β-carbons, as shown by Fuganti *et al.*[15] for cinnamaldehyde, we performed reduction of **3** by the spray dried yeast in ^2H_2O. Trideuterated product **10a** was isolated, revealing that both ^2H atoms added to the C=C bond were first incorporated into the coenzyme and then delivered to the substrate.



Another recent paper seems pertinent to our consideration; it reports on the b.y. catalyzed »hydride reduction of an epoxyde moiety«.[28] The authors have proposed the mechanism of reduction of a *para*-nitrophenyl epoxyde due to »a novel enzyme catalyzed hydride transfer from a cofactor such as NADH or NADPH«, where activated (protonated) epoxyde is opened by the hydride ion attack.

In conclusion, the overall mechanism of **1–7** could be outlined as in Scheme 4. We have demonstrated that the b.y. reduction of the carbonyl group in 2-methyl-cinnamaldehydes **1–7** is controlled by the electronic properties of *para*-substituent; reduction of the C=C bond also seems to be governed by the general shape, polarity, and hydrophilic properties of the molecule. Finally, since protonation at the α-carbon atom of enones creates a new chiral centre, there is only formal similarity between the two stereochemical models, the classic one proposed for prochiral ketones by Prelog[1] and a more recent one proposed for the C=C double bond reduction in enoates.[6,13,15] Whereas in the former case the attack of hydride ion creates a chiral (stereogenic) centre, in the latter it is the protonation of the α-carbon atom that raises such a centre. Further studies with two purified reductases are needed to explain their interplay in the microbial reduction of enones.



Scheme 4. Overall reduction mechanism of **1–7**.

EXPERIMENTAL

Melting points were determined on the Electrothermal 9100 instrument. IR spectra were obtained on a Perkin Elmer M 297 spectrometer, ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-GEM 300 spectrometer for $CDCl_3$ solutions. Shifts are given in ppm downfield from TMS as internal standard. Optical rotations were measured on the Optical Activity LTD automatic polarimeter AA-10, at ambient temperature in dichloromethane.

Compound **3** was purchased from Aldrich.

Fresh baker's yeast was obtained from Pliva, Pharmaceutical Co., Zagreb, and dry baker's yeast from Podravka Co., Koprivnica. Yeast mediated reductions at constant temperature were performed in a thermostated rotatory shaker Techtnica RVI-403.

Organic extracts were dried over Na_2SO_4 and evaporated in a Büchi rotavapor under reduced pressure.

GLC analyses were performed on a Hewlett-Packard 5890 II instrument connected to a HP 3396A integrator. HPLC analyses were performed on the HP 1050 instrument using columns with chiral support, connected to UV detector at 220 nm and HP 3395 integrator. Conditions for the separation of enantiomers, *i. e.* for determination of the optical purity of **8–14**, are given in the Table II.

para-*Dimethylamino-2-methyl-cinnamaldehyde (**1**)*

KOH (0.15 g, 2.7 mmol) was dissolved in methanol (15 mL) and freshly distilled *para*-dimethylamino-benzaldehyde (3.0 g, 20 mmol) was added. Then, freshly distilled propionaldehyde (11 mL, 150 mmol) was added under reflux dropwise over 40 h. GLC control revealed only a 55% conversion. Water (20 mL) was added and the reaction mixture was extracted with 3 × 20 mL of dichloromethane. Organic extracts were dried, evaporated, and the crude product purified by chromatography on silica gel column (200 g) with dichloromethane as eluent; 1.47 g of **1** was obtained as yellow crystals, m.p. 111–112 °C. IR(KBr) ν_{max}/cm^{-1}: 2930, 1670, 1600, 1530, 1370, 1360, 1320, 1200, 810; ^{13}C-NMR δ/ppm: 10.60, 39.67, 111.42, 122.91, 132.21, 133.21, 150.99, 151.11, 195.29; ^{1}H-NMR δ/ppm: 2.08 (s, 3H), 3.02 (s, 6H), 6.71 (d, J = 8 Hz, 2H), 7.10 (s, 1H), 7.48 (d, J = 8 Hz, 2H), 9.46 (s, 1H).

para-*Methoxy-2-methyl-cinnamaldehyde (**2**)*

Starting from *para*-methoxy-benzaldehyde (24 mL, 200 mmol), KOH (1.7 g, 30.3 mmol) was dissolved in MeOH (40 mL), and propionaldehyde (17 mL, 23 mmol) was added dropwise over 1 h at room temperature; the reaction was continued overnight. GLC control revealed a 77% conversion. Water (40 mL) was added and the reaction mixture was extracted with 3 × 50 mL of dichloromethane. After drying and evaporation of the solvent, the pure product (17.7 g, 65%, 97% assay by GLC) was obtained by distillation, b.p. 104–108 °C / 0.1 mm Hg. IR(neat) ν_{max}/cm^{-1}: 2980, 2840, 1680, 1600, 1510, 1260, 1180, 1010, 830; ^{13}C-NMR δ/ppm: 10.36, 54.91, 113.91, 127.61, 131.81, 135.73, 149.65, 160.54, 195.26; ^{1}H-NMR δ/ppm: 2.07 (s, 3H), 3.84 (s, 3H), 6.96 (d, J = 9 Hz, 2H), 7.17 (s, 1H), 7.52 (d, J = 9 Hz, 2H), 9.52 (s, 1H).

*2-Methyl-cinnamaldehyde (**4**)*

This compound was prepared as described for **2**. Pure product (18.5 g, 63%, 96.5% assay by GLC) was obtained by distillation, b.p. 131–135 °C / 14 mm Hg. IR(neat) ν_{max}/cm^{-1}: 1680, 1600, 1570, 1450, 1190, 1010, 750, 700. ^{13}C-NMR δ/ppm: 10.51, 128.52, 129.40, 129.86, 134.94, 138.11, 149.71, 195.49; ^{1}H-NMR δ/ppm: 2.06 (s, 3H), 7.25 (s, 1H), 7.35–7.53 (m, 5H), 9.57 (s, 1H).

*General Method for Preparation of **5–7***

para-Substituted benzaldehyde (50 mmol) was added to the solution of KOH (0.5 g, 8.9 mmol) in methanol (40 mL), and then propionaldehyde (5 mL, 60 mmol) dropwise over 2.5 h at room temperature. GLC control revealed a complete conversion of benzaldehyde derivatives after 3 h. On cooling the reaction solution to 0 °C, pure products crystallized and were collected by filtration.

para-*Chloro-2-methyl-cinnamaldehyde (**5**)*

3.3 g (37%) of **5** was isolated, m.p. 42–43 °C, GLC assay over 98%. IR(KBr) ν_{max}/cm^{-1}: 1680, 1590, 1190, 1010, 890, 820, 700; ^{13}C-NMR δ/ppm: 10.54, 128.82, 131.07, 133.42, 135.38, 138.56, 148.01, 195.11; ^{1}H-NMR δ/ppm: 2.05 (s, 3H), 7.21 (s, 1H), 7.44 (d, J = 8 Hz, 2H), 7.49 (d, J = 8 Hz, 2H), 9.57 (s, 1H).

para-*Cyano-2-methyl-cinnamaldehyde (**6**)*

4.6 g (58%) of **6** was isolated, m.p. 113–114 °C, GLC assay over 98%. IR(KBr) ν_{max}/cm^{-1}: 3050, 2220, 1680, 1620, 1510, 1190, 1020, 820; ^{13}C-NMR δ/ppm: 10.57, 112.37, 118.14, 130.03, 132.16, 139.24, 140.61, 146.54, 194.72; ^{1}H-NMR δ/ppm: 2.08 (s, 3H), 7.31 (s, 1H), 7.63 (d, J = 8 Hz, 2H), 7.70 (d, J = 8 Hz, 2H), 9.64 (s, 1H).

para-*Nitro-2-methyl-cinnamaldehyde (**7**)*

3.3 g (35%) of **7** was isolated, m.p. 115–117 °C, GLC assay over 98%. IR(KBr) ν_{max}/cm^{-1}: 1680, 1600, 1520, 1350, 1180, 870; ^{13}C-NMR δ/ppm: 10.60, 123.62, 130.32, 141.09, 141.19, 146.02, 147.51, 194.70; ^{1}H-NMR δ/ppm: 2.10 (s, 3H), 7.36 (s, 1H), 7.69 (d, J = 8 Hz, 2H), 8.31 (d, J = 8 Hz, 2H), 9.66 (s, 1H).

*General Method for Preparation of rac. **8–14***

para-Substituted 2-methyl-cinnamaldehydes **1–7** (2 mmol) were dissolved in ethylacetate (40 mL) and Pd/Al_2O_3 (50 mg, Aldrich) was added. Complete hydrogenation, at ambient temperature and atmospheric pressure of hydrogen, was observed (GLC) after 1.5–5 h depending on the substrate. Crude products **8–14** were separated by chromatography on silica gel column (10 g) with dichloromethane as eluent. Their enantiomers were separated on HPLC analytical columns with chiral stationary phases, at a flow rate of 0.5 ml/min, as presented in Table II. The assignment of R_t values to *R*- and *S*-enantiomers of **8–14** is based on chemical correlation of the configuration of *S*-**10** with a known compound,[29] and on the established prevalence of *S*-enantiomer in reduction of 2-methyl-cinnamaldehyde analogs.[15,25] Negative $[\alpha]_D$ values given in Table I for **8–14** also indicate their identical absolute configuration.

2-(para-Dimethylaminobenzyl)-1-propanol (*8*)

IR(neat) ν_{max}/cm^{-1}: 3350, 2910, 1615, 1520, 1350, 1030, 790; ^{13}C-NMR δ/ppm: 16.31, 37.74, 38.54, 40.68, 67.67, 112.85, 128.65, 129.67, 149.07; ^{1}H-NMR δ/ppm: 0.90 (d, J = 8 Hz, 3H), 1.84–1.91 (m, 1H), 2.34 (dd, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 2.61 (dd, J_1 = 14 Hz, J_2 = 6Hz, 1H), 2.90 (s, 6H), 3.14 (dd, J_1 = 10 Hz, J_2 = 6 Hz, 1H), 3.52 (dd, J_1 = 10 Hz, J_2 = 6 Hz, 1H), 7.69 (d, J = 8 Hz, 2H), 7.04 (d, J = 8 Hz, 2H).

TABLE II

Conditions for HPLC separation of enantiomers of **8–14**.

Comp.	X	Column	Eluent: 2-propanol/ *n*-hexan	$\frac{R_t(R)/R_t(S)}{\text{min}}$
8	$N(CH_3)_3$	Chiralpak AS	10 : 90	18.89/14.50
9	OCH_3	Chiralpak AS	10 : 90	17.48/15.34
10	$C(CH_3)_3$	Chiralcel OD	2 : 98	24.15/25.82
11	H	Chiralpak AS	10 : 90	12.82/11.47
12	Cl	Chiralpak AS	10 : 90	12.08/11.32
13	CN	Chiralpak AS	10 : 90	34.37/31.96
14	NO_2	Chiralcel OB-H	10 : 90	28.96/27.71

*2-(*para-*Methoxybenzyl)-1-propanol (**9**)*

IR(neat) ν_{max}/cm^{-1}: 2270, 2920, 1615, 1515, 1250, 1180, 1040, 800; ^{13}C-NMR δ/ppm: 16.25, 37.72, 55.06, 67.42, 113.51, 129.84, 132.49, 157.63; ^{1}H-NMR δ/ppm: 0.90 (d, J = 8 Hz, 3H), 1.86–1.93 (m, 1H), 2.37 (dd, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 2.68 (dd, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3.46 (dd, J_1 = 11 Hz, J_2 = 6 Hz, 1H), 3.52 (dd, J_1 = 10 Hz, J_2 = 6 Hz, 1H), 6.83 (d, J = 8 Hz, 2H), 7.09 (d, J = 8 Hz, 2H).

*2-(*para-tert-*Butylbenzyl)-1-propanol (**10**)*

IR(neat) ν_{max}/cm^{-1}: 3350, 2960, 1460, 1370, 1040; ^{13}C-NMR δ/ppm: 16.30, 31.15, 37.49, 38.94, 67.50, 125.04, 128.69, 137.43, 148.59; ^{1}H-NMR δ/ppm: 0.91 (d, J = 6 Hz, 3H), 1.30 (s, 9H), 1.87–1.95 (m, 1H), 2.38 (dd, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 2.70 (dd, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3.44 (dd, J_1 = 11 Hz, J_2 = 6 Hz, 1H), 3.52 (dd, J_1 = 10 Hz, J_2 = 6 Hz, 1H), 7.10 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H).

*2-(Benzyl)-1-propanol (**11**)*

IR(neat) ν_{max}/cm^{-1}: 3350, 3020, 2920, 1600, 1495, 1460, 1030, 740, 700; ^{13}C-NMR δ/ppm: 16.20, 37.56, 39.49, 67.48, 125.82, 128.21, 129.09, 140.61; ^{1}H-NMR δ/ppm: 0.91 (d, J = 8 Hz, 3H), 1.90–1.97 (m, 1H), 2.41 (dd, J_1 = 13 Hz, J_2 = 8 Hz, 1H), 2.75 (dd, J_1 = 13 Hz, J_2 = 6 Hz, 1H), 3.46 (dd, J_1 = 11 Hz, J_2 = 6 Hz, 1H), 3.52 (dd, J_1 = 11 Hz, J_2 = 6 Hz, 1H), 7.15–7.37 (m, 5H).

*2-(*para-*Chlorobenzyl)-1-propanol (**12**)*

IR(neat) ν_{max}/cm^{-1}: 3350, 2920, 1600, 1490, 1090, 1030, 1010, 790; ^{13}C-NMR δ/ppm: 16.06, 37.44, 38.68, 67.05, 128.10, 130.27, 131.34, 138.89; ^{1}H-NMR δ/ppm: 0.86 (d, J = 8 Hz, 3H), 1.82–1.93 (m, 1H), 2.35 (dd, J_1 = 13 Hz, J_2 = 8 Hz, 1H), 2.72 (dd, J_1 = 13 Hz, J_2 = 6 Hz, 1H), 3.34–3.50 (m, 2H), 7.07 (d, J = 8 Hz, 2H), 7.22 (d, J = 8 Hz, 2H).

*2-(*para-*Cyanobenzyl)-1-propanol (**13**)*

IR(neat) ν_{max}/cm^{-1}: 3440, 2930, 2220, 1620, 1510, 1030, 850; ^{13}C-NMR δ/ppm: 15.87, 37.17, 39.36, 66.78, 109.45, 118.97, 129.86, 131.93, 146.61; ^{1}H-NMR δ/ppm: 0.89 (d, J = 8 Hz, 3H), 1.88–2.01 (m, 1H), 2.46 (dd, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 2.88 (dd, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3.49 (d, J_1 = 6 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 7.56 (d, J = 8 Hz, 2H).

*2-(*para-*Nitrobenzyl)-1-propanol (**14**)*

IR(neat) ν_{max}/cm^{-1}: 3400, 3920, 1600, 1520, 1350, 1110, 750, 700; ^{13}C-NMR δ/ppm: 16.02, 37.30, 39.16, 66.87, 123.30, 128.61, 129.74, 148.64; ^{1}H-NMR δ/ppm: 0.91 (d, J = 8 Hz, 3H), 1.93–2.07 (m, 1H), 2.52 (dd, J_1 = 14 Hz, J_2 = 9 Hz, 1H), 2.93 (dd, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3.52 (d, J_1 = 6 Hz, 2H), 7.34 (d, J = 9 Hz, 2H), 8.15 (d, J = 9 Hz, 2H).

*Preparation of **22**, **23**.*

Compound **3** or **6** (3 mmol) was dissolved in MeOH (5 mL) Pd/Al_2O_3 (25 mg) and 25 mg $Ca(OH)_2$ was added. Hydrogenation was performed in a closed system at room temperature and atmospheric pressure of H_2. GLC monitoring revealed complete hydrogenation after 4 h for **3**, and after 6 h for **6**. Saturated aldehydes were separated from smaller quantities of saturated alcohols on silica gel column (10 g) with dichloromethane as eluent.

*3-(*para-tert-*Butylphenyl)-2-methyl-propanal (**22**)*

This compound was isolated in 93% yield (0.61 mg); GLC assay over 98%. IR(neat) ν_{max}/cm^{-1}: 2970, 1740, 1515, 1460, 1365, 1270, 1110, 840; ^{13}C-NMR δ/ppm: 12.9, 31.1, 34.1, 35.8, 47.2, 125.3, 128.6, 135.6, 149.1, 204.5; ^{1}H-NMR δ/ppm: 1.13 (d, J = 7 Hz, 3H), 1.36 (s, 9H), 2.58–2.76 (m, 2H), 3.10 (dd, J_1 = 13 Hz, J_2 = 5 Hz, 1H), 7.15 (d, J = 8 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 9.76 (s, 1H).

*3-(*para-*Cyanophenyl)-2-methyl-propanal (**23**)*

This compound was isolated in 79.4% yield (0.41 mg); GLC assay over 97%. IR(neat) ν_{max}/cm^{-1}: 2970, 2220, 1750, 1610, 1500, 1180, 850; ^{13}C-NMR δ/ppm: 12.72, 35.91, 47.06, 109.89, 118.54, 129.61, 131.95, 144.59, 203.10; ^{1}H-NMR δ/ppm: 1.12 (d, J = 7 Hz, 3H), 2.63–2.77 (m, 2H), 3.17 (dd, J_1 = 12 Hz, J_2 = 5 Hz, 1H), 7.32 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 9.71 (s, 1H).

Reduction of para-*Substituted 2-Methyl-cinnamaldehydes **1–7** by Baker's Yeast*

D-Glucose (5 g) was dissolved in distilled water (100 mL) and the resulting solution was thermostated at 20 °C or 30 °C. After 0.5 h fresh b.y. (10 g, *ca.* 28% dry subst.) was added, and after 1 h shaking or mixing substrate **1–7** (1.5 mmol) was added. Reaction course was followed by GLC. Samples (2 mL) were taken at regular time intervals, extracted with 2 × 4 ml of ethylacetate, dried, evaporated, and the residual material was dissolved in *ca* 1 mL dichloromethane. The solution was filtered through Millipore filter, evaporated, and dissolved in 0.2 ml of fresh dichloromethane. Samples (2 μl) were injected in GLC. Chromatographic conditions for monitoring the conversion of a single substrate are given in Table III.

The reaction was terminated by extraction with ethylacetate (2 × 200 mL). Extracts were dried, evaporated, and the crude product mixture was separated on silica gel column (10 g), with dichloromethane as eluent. Optical purity of alcohols **8–14** was determined on columns with chiral stationary phases, as presented in Table II.

*1,2,3-^{2}H-3-(*para-tert-*Butylphenyl)-2-methyl-propanol (**10a**)*

Dry baker's yeast (3 g) was slurried in $^{2}H_2O$ (50 ml 98% ^{2}H content, Fluka) and **3** (123 mg, 0.6 mmol) was added. After *ca.* 60% conversion was recorded, the reaction mixture was extracted with dichloromethane (3 × 50 mL), organic extracts were

TABLE III

Conditions for GLC monitoring of reduction of **1–7**.

Comp.	Column	Column dimension	Conditions
1	HP 5	25 m × 0.32 mm × 0.52 mm	170 °C 2 min 10 °C/min do 210 °C
2	HP 20	25 m × 0.32 mm × 0.3 mm	210 °C 5 min 10 °C/min do 220 °C
3	HP 5	25 m × 0.32 mm × 0.52 mm	180 °C (isothermal)
4	HP 20	10 m × 0.53 mm × 1.33 mm	150 °C 2 min 5 °C/min do 220 °C
5	HP 20	25 m × 0.32 mm × 0.3 mm	160 °C 2 min 10 °C/min do 220 °C
6	HP 17	10 m × 0.53 mm × 2 mm	170 °C 2 min 10 °C/min do 260 °C
7	HP 17	10 m × 0.53 mm × 2 mm	170 °C 2 min 10 °C/min do 260 °C

dried, evaporated and purified on silica gel column (10 g) with dichloromethane/cyclohexane (6 : 4) as eluent. 37 mg of pure **10a** was obtained (GLC assay 94%).

^{13}C-NMR δ/ppm: 16.17, 31.19, 34.13, 38.36 (t), 39.35 (t), 67.52 (t), 125.09, 128.72, 137.43, 148.64; ^{1}H-NMR δ/ppm: 0.90 (s, 3H), 1.30 (s, 9H), 2.67 (s, 1H), 3.50 (s, 1H), 7.17 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H).

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SAŽETAK

Studij enantioselektivne redukcije *para*-supstituiranih 2-metil-cinamaldehida pekarskim kvascem

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Redukcijom serije *para*-supstituiranih 2-metil-cinamaldehida **1–7** pri 20 °C i pH = 2–3 dobiveni su *S*-enantiomeri zasićenih alkohola **8–14** u iskorištenju od 20–80% i enantiomernom višku (e.v.) 75 – ≥ 99%; pri 30 °C niža su iskorištenja i e.v. Relativne brzine nastajanja alilnih alkohola **15–21**, katalizirane alkohol-dehidrogenazom (ADH), u korelaciji su sa Hammettovim σ^+-vrijednostima *para*-supstituenata, što pokazuje da efikasnija delokalizacija pozitivnog naboja na karbonilnom atomu usporava brzinu redukcije, dok između elektronskih svojstava supstituenata i brzine redukcije dvostruke veze, koju katalizira enoat-reduktaza, korelacije nema. Redukcijom spoja **3** u ^2H_2O sa suhim pekarskim kvascem nastaje produkt **10a** koji na α,β-ugljikovim atomima nosi atome ^2H, što je u skladu sa prethodno objavljenim rezultatima hidrogenacije selektivno ^2H-obilježenog cinamaldehida i cinamola. Prikupljeni podaci pokazuju da mehanizam redukcije C=C veze uključuje u prvom stupnju nukleofilni napad hidridnog iona na β-ugljikov atom, nakon čega slijedi enantioselektivna protonacija α-ugljikova atoma.