Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 34 (2006) 158–166
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Hydrogen transfer pathways of the asymmetric reduction of α,β-unsaturated ketone mediated by baker's yeast

Yuan Chu [a], Ben Li Zhang [b,*], Virginie Silvestre [b], Jin Pei Cheng [a,*]

[a] *Department of Chemistry, State Key Laboratory on Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China*
[b] *Université de Nantes, Laboratoire d'Analyse Isotopique et Electrochimique de Métabolismes, CNRS UMR 6006, 2, rue de la Houssinière, 44322 Nantes, France*

Received 9 March 2006

Abstract

The hydrogen transfer mechanism of cofactor reduction and recycling processes in the yeast reduction of α,β-unsaturated ketone was studied by using quantitative isotope tracing close to natural abundance measured by ^{2}H NMR. In the reaction, the active cofactor is NADPH. The cofactor-transferred hydride attacks the β sp^2 carbon of the enone carbonyl while water hydrogen is transferred to the α position. The reductant involved in the reaction depends on the quantity of yeast. When the amount of yeast is very large, the enzymes use preferentially certain unidentified substance stored in the yeast cells rather than the added glucose as electron donor. In this case, the hydrogen transferred by the cofactor is mainly of water origin. When the yeast amount is low, the added glucose is more efficiently used by the enzymes as electron donor and its hydrogen atoms bound to C-1 and C-3 are delivered to the substrate.

Keywords: Biocatalysis; Asymmetric bioreduction; Cofactor recycling; NADPH; Isotope tracing; Baker's yeast; Deuterium NMR

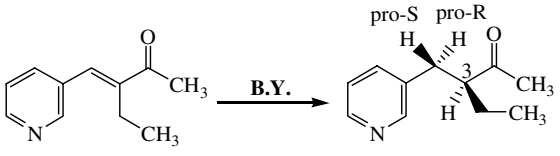
* Corresponding authors. Fax: +33 251125712 (B.L. Zhang).
E-mail address: benli.zhang@univ-nantes.fr (B.L. Zhang).

0045-2068/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2006.04.002

1. Introduction

Microbial reduction of unsaturated compounds is a facile and green process for organic synthesis of chiral compounds. Among the microorganisms suitable for the biotransformation, baker's yeast (*Saccharomyces cerevisiae*) is the most useful since it is cheap and readily available and can catalyze a wide range of reactions [1]. Baker's yeast-mediated biotransformation holds great potential for the industrial production of enantiopure compounds [2]. In the asymmetric reduction with baker's yeast, the cofactor NAD(P)H plays an important role in acting as a porter to capture a hydride from a reductant and deliver it later to a substrate. In order to better apply this methodology to a broader scope of reactions and to better manipulate the working conditions, it is desirable to study the hydrogen transfer mechanisms between the reductant and the cofactor as well as between the cofactor and the substrate. Though there are already numerous studies on the microbial reduction mechanism, especially the stereochemistry of the steps involving the cofactor, few works on the hydride transfer pathways from reductant to the cofactor were reported to our best knowledge. In fact, in the yeast reduction, the substrate to be reduced is obviously the oxydant, but what is the reductant (electron donor) is not clear [3,4]. Thus, more studies on the cofactor regeneration mechanism are necessary in order to understand how the hydride is transferred from reductant to the cofactor. Isotope labeling is very useful in this regard [4–7]. The asymmetric saturation of carbon–carbon double bond of α,β-unsaturated ketone (enone) is an important baker's yeast-mediated reaction. The stereochemistry of this double bond saturation has been studied by Fronza et al. using isotope labeling [6,8–10]. In the present work, we studied the hydrogen transfer mechanism of both cofactor reduction and recycling processes of this reaction by using a special technique: quantitative isotope tracing close to natural abundance measured by ^{2}H NMR [11,12]. Through fine quantitative analysis of the results of simple labeling experiments, the technique can provide more information on deuterium transfer mechanism. The technique has been applied to the study of hydrogen transfer pathway of several complex biotransformation systems [11–14]. The mechanism of asymmetric yeast reduction of the carbonyl of acetoacetate has also been studied in this way [4] and it was found that the hydride was transferred to the carbonyl carbon from the cofactor NADPH and the hydrogen was provided by the added glucose through hexose monophosphate pathway (HMP) or certain unidentified substances stored in the yeast cells if no exogenous electron donor was added. In order to elucidate the hydride transfer pathway during the carbon–carbon double bond saturation, we chose the reduction of (*E*)-3-ethyl-4-(3-pyridyl)-3-buten-2-one (**1**) as the model reaction since in the reaction, the further reduction of the carbonyl is negligible and both the yield and the optical purity of the product, (*S*)-3-(3-pyridylmethyl)-2-pentanone (**2**), are very high [15].

Graphic 1



1 **2**

The parameter measured by ^2H NMR [16] is the ratio of the number of deuterium (D) atoms to that of protium (H) atoms at site i of the sample molecule, $(D/H)_i$ in ppm:

$$(D/H)_i = N_{D_i}/(P_i N_H)$$

where N_{D_i}: number of site i deuterated isotopomers, P_i: the stoichiometric hydrogen number at site i, (for example, for $-CH_3$, $P_i = 3$), N_H: number of unlabeled (totally protiated) molecules which is approximately equal to the number of all sample molecules containing different isotopomers at natural abundance. $(D/H)_i$ of all sites of the sample compound can be determined when their ^2H NMR signals are well resolved.

2. Materials and methods

2.1. Baker's yeast reduction of (*E*)-3-ethyl-4-(3-pyridyl)-3-buten-2-one

3-Ethyl-4-(3-pyridyl)-3-buten-2-one was synthesized according to the literature [15]. The reference glucose (produced from corn) was obtained from Prolabo. The [1-$^2H_1$]glucose were purchased from Aldrich. The [3-$^2H_1$]glucose and [4-$^2H_1$]glucose were purchased from Omicron. The isotopic purity of these isotopically substituted glucoses molecules is at least 97% determined by NMR. D_2O (>99.8%) was purchased from Beijing chemical works. The dried yeast of *Saccharomyces cerevisiae* was purchased from Val-Oeno. Number of living cells: 2.4 10^{10}/g, number of wild cells: absent, bacteria number: 3.9 10^5/g.

Glucose slightly enriched at specific positions were prepared by adding small quantity of the specifically deuterium-substituted glucose (≈10 mg) to 37.5 g of reference corn glucose dissolved in 450 mL of tap water in the first set experiment and to 15 g of reference corn glucose dissolved in 750 mL tap water in the second set experiments. The $\Delta(D/H)_{j\text{-glucose}}$ value in ppm was evaluated with the following equation:

$$\Delta(D/H)_{j\text{-glucose}}(\text{ppm}) = \frac{(\text{mass of site j deuterated glucose} \times f)/181}{\text{mass of reference glucose}/180} \times 10^6$$

where f is the isotopic purity of the deuterated glucose determined by ^1H NMR. The deuterium-enriched medium water was prepared by adding small amount of D_2O to tap water. Its (D/H) value was determined by ^2H NMR.

In the first set experiment, the composition of the fermentation medium in each 500 mL flask was: substrate (**1**) (0.5 g), reference glucose or its mixture with labeled glucose (12.5 g), baker's yeast (25.0 g) and tap or enriched water (150 mL). Three or four flasks containing the reaction mixture plugged with cotton were shaken for 24 h in the shaking box at 25 °C. The reaction was monitored by gas chromatography and continued until the conversion rate of the reaction was above 95%. The fermentation medium in the flasks was combined and then centrifuged to remove the biomass. The solution was extracted by dichloromethane. The extract was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel. The purified product (**2**) was obtained as a yellow oil. Yield >90%. The spectral and physical data of the final product of one sample were listed as following: (*S*)-enantiomer; ^1H NMR (C_6D_6, TMS) δ 0.65 (3H, t, $J = 6.3$ Hz), 1.10–1.47 (2H, m), 1.60 (3H, s), 2.08–2.40 (2H, m), 2.55–2.68 (1H, m), 6.72–6.77 (1H, m), 7.02–7.12 (1H,

m), 8.42–8.49 (2H, m); $[\alpha]_D^{23} + 11.1$ (*c* 5.39, EtOH). Found: C, 74.36%; H, 8.61%; N, 7.75%. Calculated for $C_{11}H_{15}NO$: C, 74.54%; H, 8.53%; N, 7.90%.

In the second set of experiments, the composition of the fermentation medium in each 500 mL flask was: substrate (**1**) (0.5 g), reference glucose or its mixture with labeled glucose (3.0 g), baker's yeast (3.0 g), and water (150 mL). For each reduction, five flasks were used. The procedure was the same as described above.

2.2. ^2H NMR measurement

For ^2H NMR measurement [4,16] of **2**, about 1.5 g of sample was mixed with 0.1 mL of dioxane (external reference) and 0.065 mL of C_6F_6 (lock material) and then dissolved in 1.5 mL of benzene. The sample solution was filtered into a 10 mm diameter NMR tube. The deuterium NMR spectra were recorded at 76.77 MHz under broad-band proton decoupling using a Bruker DRX 500 spectrometer equipped with a ^{19}F lock device. Other conditions were: temperature 315 K, O1 400 Hz, O2 2200 Hz, frequency window 1497 Hz, memory size 16K, acquisition time 3.4 s, scan number 6000, and an exponential multiplication corresponding to a line broadening of 0.5 Hz. Three spectra were recorded for each sample and an average $(D/H)_i$ value was calculated from the three measurements.

The $(D/H)_i$ values were determined using the external reference dioxane of which the isotopic ratio $(D/H)_R$ was precisely determined by ^2H NMR. $(D/H)_i$ was calculated from the following equation:

$$(D/H)_i = (D/H)_R P_R m_R M_S S_i / (P_i f m_S M_R S_R)$$

where P_i and P_R are the stoichiometric hydrogen numbers in site i and in the reference. M_S, m_S, M_R and m_R are the molecular weight and mass of the sample and the reference, respectively. f is the purity of the sample in mole fraction determined by GC; S_i and S_R are, respectively, surface area of the signals of the site i monodeuterated molecule and the reference in the ^2H NMR spectrum. The quantitative evaluation of the surface areas was performed by using a curve-fitting program (PERCH solutions Ltd., FIN-70211, Kuopio, Finland).

3. Results and discussion

In this work, two sets of reduction experiments were carried out. In each set, the substrate was added to a medium containing only dry baker's yeast and glucose in water. The labeling was performed at the level of water and glucose. The substrate was at first reduced in tap water with unlabeled glucose (reference), then it was reduced in labeled water prepared by adding very small amount of D_2O in tap water and in tap water but successively with C-1, C-3 and C-4 (only for the first set) labeled glucose (the labeling was realized by adding very small amount of the specifically labeled glucose to the unlabeled glucose). The (D/H) ratio of tap and labeled water were determined by ^2H NMR. For water labeling, the solvent (reaction medium) hydrogen isotope ratios $(D/H)_m$ were used. They were evaluated in taking into account the presence of exchangeable hydrogen of the glucose [12,13]. The glucose labeling was quantified by $\Delta(D/H)_{j\text{-glucose}}$ (with j = 1, 3, and 4) which is the increment of the hydrogen isotope ratio of a given site j of the glucose due to addition of the specifically labeled glucose. The $\Delta(D/H)_{j\text{-glucose}}$ values were calculated on the basis

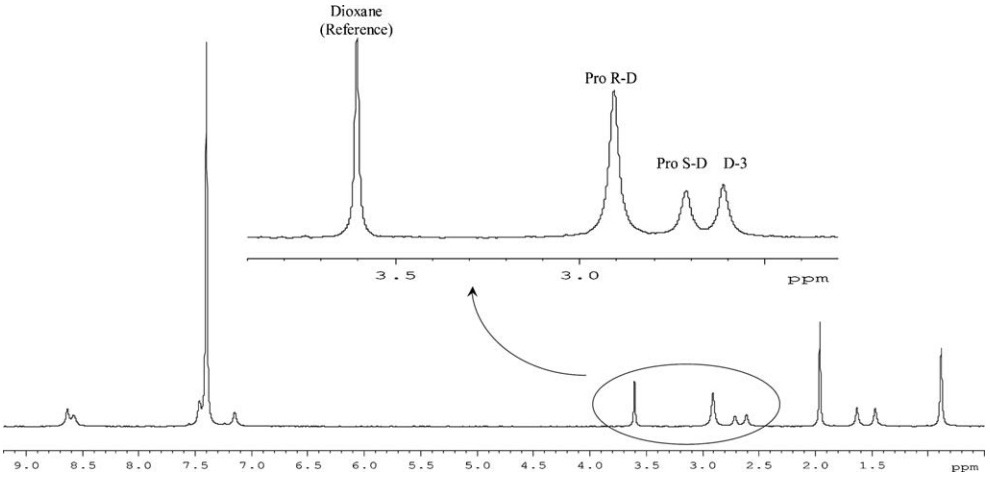


Fig. 1. ^{2}H NMR spectrum in C_6D_6 of the product (**2**) obtained in tap water.

of mass of labeled and unlabeled glucose [11]. The product (**2**) samples obtained with different labelings, after isolation and purification, were analyzed by ^{2}H NMR (Fig. 1) in order to determine their $(D/H)_i$. We are particularly interested by the two hydrogen atoms introduced during the reduction: the pro *S* hydrogen of the methylene between the pyridyl and C-3 (pro *S* and pro *R* were assigned only on the basis of anti-addition) [8–10,17] and the C-3 hydrogen.

According to the method of quantitative isotope tracing close to natural abundance, [4,11,12] for each site i of the product, a linear equation can be established between $(D/H)_i$ and $(D/H)_m$ of the medium:

$$(D/H)_i = a(D/H)_m + b$$

The equations were determined on the basis of $(D/H)_i$ and $(D/H)_m$ for samples obtained in tap water and enriched water. The magnitude of slope a reflects the contribution of deuterium of water to $(D/H)_i$ of the product and the intercept b represents the contribution of unexchangeable hydrogen, especially the carbon-bound hydrogen atoms of the electron donor. In a similar way, on the basis of the values of $(D/H)_i$ and $\Delta(D/H)_{j\text{-glucose}}$ obtained for the reference (unlabeled glucose in tap water) and labeling experiments (unlabeled glucose + site j enriched glucose in tap water), we obtained

$$(D/H)_i = a'\Delta(D/H)_{j\text{-glucose}} + b'$$

The slope a' reflects the contribution of deuterium of site j of glucose to $(D/H)_i$ of the product.

In the first set of reactions, large amount of yeast and glucose were used. The reduction was performed under the following conditions: in each flask (with 3 or 4 flasks), substrate (0.5 g), yeast (25 g), glucose (12.5 g), and solvent (water) (150 mL). The substrate/dry yeast ratio was very close to that of reference 15, but in their work, no glucose was added. With the data of Table 1, we obtained the following relations:

Table 1
Isotopic data of samples obtained in the first set of experiments with high amount of yeast and glucose

Labeling	e.e. (%)	$(D/H)_w$ (ppm)	$(D/H)_m$ (ppm)	$\Delta(D/H)_{j\text{-glucose}}$ (ppm)	$(D/H)_{proR}$[a] (ppm)[b]	$(D/H)_3$ (ppm)[b]	$(D/H)_{proS}$[a] (ppm)[b]
Tap water[c] (reference)	84	149.5	149.7	0	279.6(0.7)	91.8(0.6)	87.7(2.2)
^{2}H enriched water[c]	94	328.2	324.8	0	291.6(1.9)	189.2(1.1)	147.7(1.6)
[4-^{2}H]glucose[d]	79	149.5	149.7	283	281.1(6.4)	84.7(1.0)	79.7(1.9)
[1-^{2}H]glucose[d]	85	149.5	149.7	278	283.2(2.5)	88.0(2.6)	94.4(1.9)
[3-^{2}H]glucose[d]	96	149.5	149.7	279	278.2(1.4)	87.5(1.9)	103.8(0.6)

[a] The hydrogen of the methylene between the pyridyl and C-3 of **2**.
[b] In parentheses are standard deviations of NMR analysis.
[c] With the corn glucose.
[d] Mixed with the corn glucose in tap water.

$$(D/H)_3 = 0.56(D/H)_m + 8.5 \quad (1)$$

$$(D/H)_{proS} = 0.34(D/H)_m + 36.4 \quad (2)$$

$$(D/H)_{proS} = 0.02\Delta(D/H)_{1\text{-glucose}} + 87.7 \quad (3)$$

$$(D/H)_{proS} = 0.06\Delta(D/H)_{3\text{-glucose}} + 87.7 \quad (4)$$

During the double bond saturation, one of the pyridyl-bound methylene hydrogen of **2** is delivered by NADPH as hydride and that of C-3 is picked up from water. This view was supported by the reduction performed using purified yeast extract in D_2O in the presence of NADPH and in H_2O in the presence of specifically labeled NADPD, respectively [6]. Our results confirm this conclusion in a different way. The slope of Eq. (1) is large while its intercept is very small. It should be noted that Eq. (1) was obtained based on the $(D/H)_3$ value of the first line (91.8). However, the measurement of $(D/H)_i$ of big molecules is less precise than small molecule and the results may exhibit some fluctuation. This value is the highest limit among other similar values since theoretically, the value should be very close for all samples obtained in tap water even with labeled glucose. If we use the average value of $(D/H)_3$ of all samples prepared in tap water in the same column of Table 1, the Eq. (1) can be $(D/H)_3 = 0.56(D/H)_m + 1.5$. That means the C-3 hydrogen of **2** come only from water. The pro *S* pyridyl-bound methylene hydrogen was transferred by the cofactor since the slope of Eq. (2) is smaller than that of Eq. (1) and its intercept is not negligible. This is because the NADPH-transferred deuterium atoms come from both the medium and the unexchangeable sites of the electron donors [4,14]. In enriched water, the $(D/H)_{proR}$ value of **2** also increased slightly. This may be due to the formation of a small amount of its *R*-isomer. The $(D/H)_{proS}$ value did not change when C-4 hydrogen of glucose was labeled. This result confirms that the cofactor is NADPH and not NADH because the regeneration of NADH can be performed through the glycolysis pathway in which it transfers the hydrogen of medium and that bound to C-4 of glucose [4,12]. In the yeast cell, NADPH can be regenerated through the HMP pathway and the source of the transferred hydrogen is the unexchangeable hydrogen of C-1 and C-3 of glucose and, to a lower degree, the medium, so that when C-1 and C-3 labeled glucose was used, $(D/H)_{proS}$ increased (Table 1, Eqs. (3) and (4)). The slope of Eq. (4) is comparatively higher the that of Eq. (3), in accordance with our previous observation that more H atoms of C-3 of glucose were transferred than those of C-1 of glucose[4,14]. But the slopes of both Eq. (3) (0.02) and Eq. (4) (0.06) are very small. These results show that few hydrogen atoms were provided

by glucose and that the enzymes used the electron donors stored in the yeast cells rather than the added glucose. But the cell-stored reductant should not be glucose which is the main constituent of the carbohydrates stored in yeast cell, because the slope of Eq. (2) is too high and its intercept is too low. Since the slope value of Eq. (2) (0.34) is quite large, a considerable part of the cofactor-delivered hydrogen atoms must come from water as was observed in the yeast reduction of acetoacetate in the absence of glucose [4]. How the large amount of water hydrogen atoms were transferred to the cofactor in these cases is not known.

The NAD(P)H can exchange its active hydrogen with water indirectly via flavin catalyzed by diaphorase and this reaction can explain partly the presence of water hydrogen in the cofactor. But the isotope tracing study on the exchange and alcohol fermentation performed with baker's yeast show that this exchange is quite limited [12]. In addition, in the study of yeast reduction of acetoacetate, [4] two sets of water labeling experiments were carried out using the same amount of yeast (weight yeast/substrate ratio is 16/5): one was performed with added glucose and the other without added reductant. The degree of diaphorase-catalyze exchange should be the same in the two cases. However, it was observed that when glucose was added, the cofactor-transferred hydrogen come mainly from glucose (small slope, 0.17 and large intercept, 85.2) while in absence of glucose it come mainly from water (large slope, 0.45 and small intercept, 7.5). In the latter case, the slope value (0.45) is higher than that of Eq. (2) (0.34) even though the weight yeast/substrate ratio in the experiments resulting in Eq. (2) is much higher (25/0.5), because glucose was added. These results show that the large amount of water hydrogen in NADPH should not be only due to the diaphorase-catalyzed exchange and that the variation of yeast amount should not modify the degree of the exchange because the NAD(P)H/diaphorase/flavin ratio in the yeast is constant. On the basis of the results of the first set of experiments in the present work, it can be concluded that when the amount of yeast is in large excess, the use of added glucose as electron donor by the yeast enzymes is not efficient even if the sugar amount is plenty.

In the second set of experiments, we substantially reduced the quantity of yeast and added less glucose in the medium. The reduction was performed under the following conditions: in each flask (with 5 flasks), substrate (0.5 g), yeast (3 g), glucose (3 g), and solvent (water) (150 mL). With the data of Table 2, we obtained the following relations:

Table 2
Isotopic data of samples obtained in the second set of experiments with low amount of yeast and glucose

Labeling	e.e. (%)	$(D/H)_w$ (ppm)	$(D/H)_m$ (ppm)	$\Delta(D/H)_{j\text{-glucose}}$ (ppm)	$(D/H)_{proR}$[a] (ppm)[b]	$(D/H)_3$ (ppm)[b]	$(D/H)_{proS}$[a] (ppm)[b]
Tap water[c] (reference)	87	149.5	149.7	0	319.6(1.8)	101.7(1.2)	114.5(1.8)
^{2}H enriched[c] water	95	328.2	324.8	0	286.7(1.6)	219.9(2.4)	137.1(6.3)
[1-^{2}H]glucose[d]	82	149.5	149.7	644	295.5(7.0)	102.7(1.1)	154.6(3.1)
[3-^{2}H]glucose[d]	82	149.5	149.7	661	301.0(8.0)	95.3(3.4)	213.1(5.7)

[a] The hydrogen of the methylene between the pyridyl and C-3 of **2**.
[b] In parentheses are standard deviations of NMR analysis.
[c] With the corn glucose.
[d] Mixed with the corn glucose in tap water.

$$(D/H)_3 = 0.68(D/H)_m + 0.7 \quad (5)$$

$$(D/H)_{proS} = 0.13(D/H)_m + 95.2 \quad (6)$$

$$(D/H)_{proS} = 0.06\Delta(D/H)_{1\text{-glucose}} + 114.5 \quad (7)$$

$$(D/H)_{proS} = 0.15\Delta(D/H)_{3\text{-glucose}} + 114.5 \quad (8)$$

Some of the ^{2}H NMR analysis results of this set are less accurate than the first set and so the uncertainty is greater. Especially, the fluctuation of $(D/H)_{proR}$ value is quite large. Although this parameter is less interesting, the influence of different e.e. values on this parameter cannot be analyzed. Eq. (5) confirms the water origin of C-3 hydrogen of **2** since its slope is high and its intercept is nearly zero. The kinetic isotope effect of the hydrogen transfer step from water to this site can also be evaluated using the slope of Eq. (5) and is equal to $0.68^{-1} = 1.5$ [12,18]. Eq. (6) shows that there are less H atoms of water (small slope: 0.13) and more unexchangeable H atoms (high intercept value: 95 ppm) at the pro *S* position. The slopes of Eqs. (7) and (8) confirm this result and indicate that more H atoms of C-1 and C-3 glucose were transferred by the cofactor to this position than in the first set. Once again we observed (Eqs. (7) and (8)) that at this position, the deuterium atoms of C-3 of glucose are more than those of C-1 [4,14]. In this case, glucose is an active reductant. Compared with the first set, even though the amounts of both yeast and glucose were reduced, the efficiency of the use of added glucose was substantially increased. These results are very useful for the choice of working conditions of this type of bioreductions.

4. Conclusion

In conclusion, in the saturation of carbon–carbon double bond of enone with baker's yeast, the active cofactor is NADPH. The cofactor-transferred hydride attacks the β sp^2 carbon of the enone carbonyl while the solvent (water) hydrogen is transferred to the α position. When the amount of yeast is very large, the enzymes use preferentially certain substances stored in the yeast cells rather than the added carbohydrate as electron donor. The cell-stored electron donors cannot be identified at the present stage. In this case, the hydrogen transferred by NADPH is mainly of water origin. When the yeast amount is low, the added carbohydrate is more efficiently used by the enzymes as electron donor. The main hydrogen source transferred by the cofactor NADPH is the hydrogen atoms bound to C-1 and, especially, C-3 of glucose since the cofactor is regenerated in the oxidative branch of the hexose monophosphate pathway.

Acknowledgments

We thank the Natural Science Foundation of China (n°20472038) and (n°20421202) who supported partly this work.

References

[1] (a) K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, Tetrahedron Asymmetry 14 (2003) 2659–2681;
(b) R. Csuk, B.I. Glanzer, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, p. 527;
(c) P. Arrigo, G. Pedrocchi-Fantoni, S. Servi, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, p. 365;

(d) P. Arrigo, G. Pedrocchi-Fantoni, S. Servi, Adv. Appl. Microbiol. 44 (1997) 81–123;
(e) K. Faber, Biotransformations in organic chemistry, third ed., Springer, Berlin, 1997;
(f) R. Csuk, B.I. Glänzer, Chem. Rev. 91 (1991) 49–97.
[2] A. Liese, K. Seelbach, C. Wandrey, Industrial Biotransformation, VCH-Wiley, Weinhein, 2000.
[3] W.F.H. Sybesma, A.J.J. Straathof, J.A. Jongejan, J.T. Pronk, J.J. Heijnen, Biocatal. Biotransfor. 16 (1998) 95–134.
[4] B.L. Zhang, S. Pionnier, Chem. Eur. J. 9 (2003) 3604–3610.
[5] F.H. Westheimer, H.F. Fisher, E.E. Conn, B. Vennesland, J. Am. Chem. Soc. 73 (1951) 2403.
[6] G. Fronza, C. Fuganti, M. Mendozza, R.S. Rallo, G. Ottolina, D. Joulain, Tetrahedron 52 (1996) 4041–4052.
[7] X.Q. Zhu, Y.C. Liu, J.P. Cheng, J. Org. Chem. 64 (1999) 8980–8981.
[8] G. Fronza, C. Fuganti, P. Grasselli, M. Barbeni, Tertahedron Lett. 33 (1992) 6375–6378.
[9] G. Fronza, C. Fuganti, P. Grasselli, A. Mele, A. Sarra, G. Allegrone, M. Barbeni, Tertahedron Lett. 34 (1993) 6467–6470.
[10] G. Fronza, C. Fuganti, P. Grasselli, S. Lanati, R. Rallo, S. Tchilibon, J. Chem. Soc. [Perkin 1] (1994) 2927–2930.
[11] B.L. Zhang, Yunianta, M.L. Martin, J. Biol. Chem. 270 (1995) 16023–16029.
[12] S. Pionnier, R. Robins, B.L. Zhang, J. Agric. Food Chem. 51 (2003) 2076–2082.
[13] S. Pionnier, B.L. Zhang, Anal. Biochem. 307 (2002) 138–146.
[14] O. Roger, R. Levigne, M. Mahmoud, C. Buisson, B. Onno, B.L. Zhang, R. Robins, J. Biol. Chem. 279 (2004) 24923–24928.
[15] Y. Kawai, M. Hayashi, N. Tokitoh, Tetrahedron Asymmetry 12 (2001) 3007–3013.
[16] M.L. Martin, G.J. Martin, in: P. Diehl, E. Fluck, H. Günther, R. Kosfeld, J. Seelig (Eds.), NMR Basic Principles and Progress, vol. 23, Springer-Verlag, Berlin, 1990, pp. 1–61.
[17] S. Okuda, GC-MS News 13 (1985) 94–100.
[18] B.L. Zhang, S. Pionnier, J. Phys. Org. Chem. 14 (2001) 239–246.