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Microbiological transformations 37. An enantioconvergent synthesis of the β-blocker (*R*)-Nifénalol® using a combined chemoenzymatic approach♦

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Abstract. This work describes the synthesis of (*R*)-Nifénalol® based on an enantioconvergent chemoenzymatic hydrolysis of *para*-nitrostyrene oxide. A mathematical approach is devised which allowed to optimize the process. © 1997 Elsevier Science Ltd.

The synthesis of optically enantiopure or vicinal diols is nowadays one of the most actively studied topics in asymmetric synthesis. This is due in particular to the fact that these compounds are highly valuable chiral synthons used in numerous syntheses of biologically active molecules. Several methodologies have been explored in order to prepare these targets, the challenge being to get them in enantiopure form as well as in high preparative yields. Some of these approaches, like for instance the use of the salen manganese or chromium Jacobsen's catalysts[1] allows direct oxidation of the olefinic precursor whereas very recently, it has been shown that some cobalt-based catalysts allow the opening of epoxides to enantiomerically enriched protected diols.[2] Some other ones, like for instance the very efficient Sharpless osmium catalyzed dihydroxylation reaction, go through the preliminary preparation of vicinal diols which can further on be cyclized without loss of stereochemical integrity into the corresponding epoxide.[3]

One of the recognized advantages of these approaches is the fact that the starting olefins are generally prochiral compounds thus leading, in principle, to a 100% reaction yield. However, this is not always verified on the practical point of view and, whereas in several cases the enantiomeric excesses (ee) of the obtained products have been shown to be good to excellent, some other substrates only led to moderate, or even poor ee. Furthermore, both these processes are based on the use of heavy metal catalysts which may be sources of industrial pollution. Also, one very serious drawback of these methodologies is the fact that they are patent-pending procedures, which implies the payment of royalties for industrial use.

♦. This work has been first presented at the BIOTRANS 95 Congress (September 95, Warwick, UK) and at the International Conference on Biotechnology for Industrial Production of Fine Chemicals (September 29-October 02, 1996, Zermatt, Switzerland). The results are part of the PhD theses of S. Pedragosa-Moreau and of C. Morisseau.

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Another appealing way to the synthesis of such chirons is to use a biocatalytic approach. In this context, different direct or indirect ways have been reviewed recently.[4,5] One of the most promising methods in this context is the use of a new emerging type of enzymes -i.e. Epoxide Hydrolases (EHs). These enzymes have been proven to be able to discriminate efficiently between pseudoasymmetric centers of *meso* substrates as well as to enantioselectively hydrolyze various racemic epoxides, thus allowing to recover the unreacted substrate in enantiopure form together with the corresponding diol which also, in many cases, is of high enantiopurity. In some cases, the different regioselectivity displayed by these enzymes for each of the substrate enantiomers may lead to the enantioconvergent synthesis of one single enantiomer which can thus be obtained in a 100% yield in theory.[6,7] Several such enzymes have been described and studied in the past few years, most of them being from mammalian origin,[8] and the mechanism implied in these reactions has been determined only recently.[9,10] However, the use of these mammalian enzymes for gram-scale preparative purposes is severely limited due to their low availability.

It has been shown recently that these enzymes also exist in plants[11] and bacteria[12] and we have focused our attention on enzymes from fungal origin, these cells being easily cultured in large scale fermentors. We thus have shown that various fungi, such as *A. niger* or *B. sulfurescens*, were able to achieve the enantioselective hydrolysis of various types of epoxides like for instance geraniol,[13] limonene[13] or styrene epoxides.[14] Furthermore, we have observed that, in the case of styrene oxide, these fungi were enantiocomplementary since *A. niger* hydrolyzed the (*R*)-enantiomer, whereas *B. sulfurescens* preferentially hydrolyzed the (*S*)-antipode. Interestingly enough, both these fungi led to the vicinal diol of the (*R*) absolute configuration, due to a different regioselectivity of the nucleophilic attack.[15] This therefore allowed us to set up a reaction implying a mixture of these two fungi which led to an enantioconvergent (theoretical 100% yield) of enantiopure diol.[14] Hovewer, depending on the substrate structure, it might happen that this approach is not usable because of the lack of such enantiocomplementary hydrolyses. In this paper, we describe the synthesis of the biologically active enantiomer of the β-blocker (*R*)-Nifénalol®**1**, in good yield in spite of the intrinsic 50% limitation implied in a resolution process.

RESULTS AND DISCUSSION

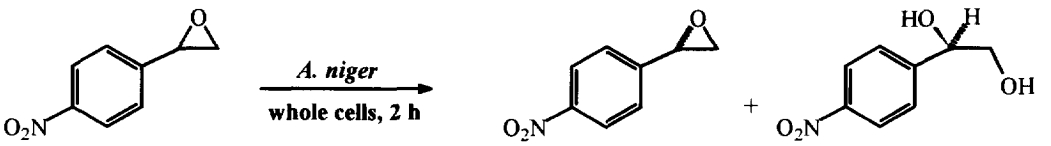
Nifénalol®**1** is a molecule endowed with interesting biological properties since it has been shown to act as a β-adrenergic blocker with antianginal and antiarhythmic properties. Furthermore, it has been emphasized that only its (*R*)-enantiomer displays biological activity.[16] This optically active form has been previously prepared *via* resolution of its D(-) dibenzoyltartric acid derivative.[17] Another tempting way to this compound was to use (*R*)-epoxide **2** as a key intermediate. Thus, we first explored the possibility to prepare this chiral synthon using our whole-cell catalyzed hydrolysis of the racemic epoxide with either *A. niger* or *B. sulfurescens*, separately.

Synthesis of the enantiopure (R)-2 epoxide.

In the course of this study, a first problem stemmed from the very low solubility of **2** in the aqueous medium. Preliminary experiments showed that several organic cosolvants could be used -i.e. DMF, DMSO, acetone, acetonitrile and ethanol, the best results being obtained with either DMSO or DMF. Therefore, our analytical experiments were conducted by adding a solution of **2** in DMF (1% v/v) into a buffered (pH 8) whole-cell suspension of the appropriate fungus. As we described previously these experiments led, when conducted with *A. niger*, to a satisfactory hydrolysis of **2**, the remaining epoxide reaching an ee value higher than 98% after 1 h (for about 30% yield), whereas the ee of the formed diol was about 70% (about 54% yield) in the same time.[18] However, when conducted with the fungus *B. sulfurescens* this hydrolysis only led to a very slow hydrolysis, about 36% of **3** (49% ee) being formed after about 24 h.[18] This disappointing result indicated that it was not possible, in this case, to prepare diol **3** in yields higher than 50% by using our previously described methodology based on the simultaneous use of the two fungi.

A first obvious way to the preparative scale synthesis of (*R*)-Nifénalol **1** was to achieve the preparation of (*R*)-**3** *via* the *A. niger* catalyzed resolution. This could be scaled up easily and performed on 5 g of racemic **2** using a 1 L buffered solution containing 40 g of cells (dry weight) obtained from a 5 L culture and a 5% DMF concentration. The reaction was monitored by chiral GC and was stopped when the ee of the remaining epoxide reached 95% (2 h). Standard work-up followed by flash chromatography led to isolation of 1.9 g (38%) of pure (*S*)-**2** and 2.7 g (49%) of diol (*R*)-**3** showing respectively ee values of 96 and 66% (Scheme 1). Interestingly, diol (*R*)-**3** could be easily recrystallized ($CHCl_3$) which led to 1.7 g (32% yield) of enantiopure material.

Scheme 1



5 g (±)-**2**

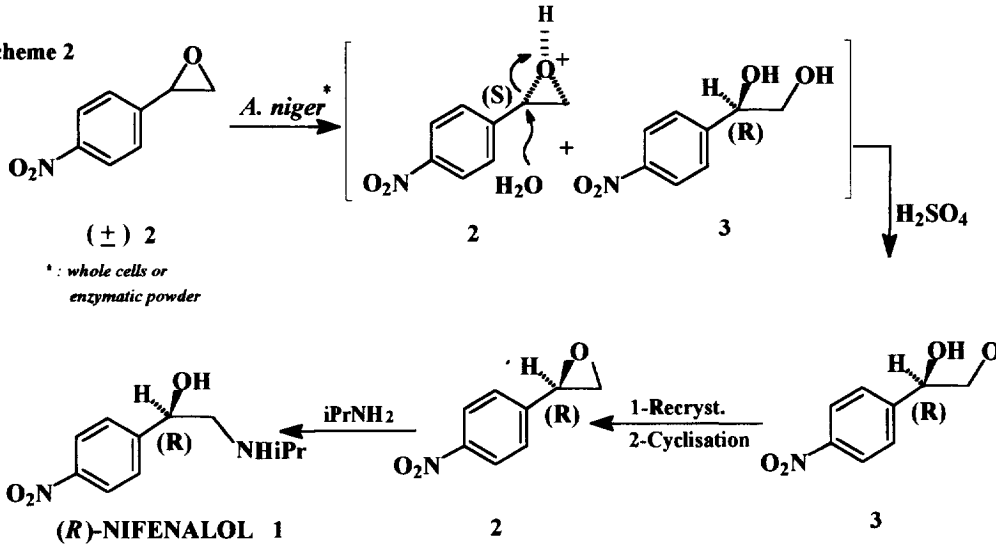
1.9 g **(*S*)-2**
ee 96%; yld 38%

2.7 g **(*R*)-3**
ee 66%; yld 49%
(recrys. ee>98%; yld 32%)

In order to overcome the loss of half of the substrate due to this resolution process, we explored the possibility of using simple acid hydrolysis of the remaining (*S*)-**2** epoxide which, assuming an inversion of configuration at the stereogenic benzylic carbon atom, could interestingly allow the preparation of the corresponding (*R*)-**3** diol from (*S*)-**2** and the transformation of the total amount of the starting material to (*R*)-Nifénalol **1**. In order to optimize the final yield of the diol (*R*)-**3** (as well as its correlated ee value after acid hydrolysis), a most appealing way was to combine the enzymatic *and* the acid-catalyzed hydrolyses in a one-pot

process (Scheme 2). However, the accurate tuning of these two consecutive reactions was obviously not intuitively predictable because the final ee of the diol would be directly dependent upon the conversion ratio. Thus, a general equation relating the value of the final diol to the conversion ratio -or to the ee of the remaining epoxide (which in fact is much easier to measure exactly using chiral GC)- was clearly needed. Interestingly, a similar approach, focused on a combinated lipase esterification/Mitsonobu's inversion, was described recently and mathematical equations which link the enzymatic and chemical steps were derived.[19] These equations were established on the base of Sih's theory[20] and allowed to calculate the E value assuming total inversion during Mitsonobu's reaction.

Scheme 2

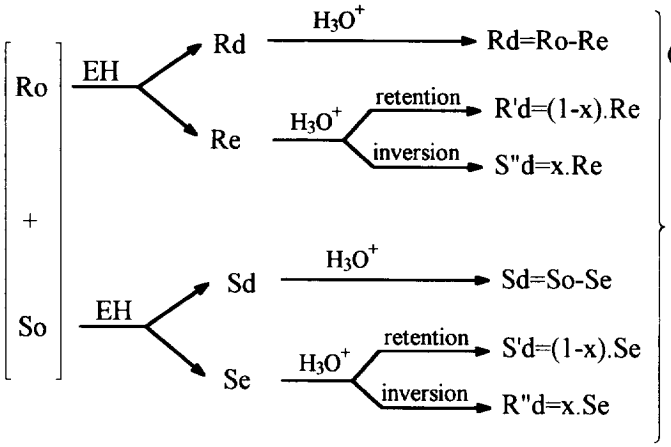


Equations

Application of these equations to the case of our combinated EH/acid catalyzed process necessitated (a) to accurately determine the E value of the enzymatic hydrolysis and (b) to control the stereoselectivity of the oxirane opening during chemical hydrolysis. Based on the ee of both the residual epoxide (ee_s) and the formed diol (ee_p) at several conversion ratios (c), we have previously determined the E value of this reaction as being about 40.[21] Concerning the stereochemistry of the acid catalyzed hydrolysis, our experiments indicated that careful acid hydrolysis of pure (*S*)-**2** with H_2SO_4 (in a DMF/water solution) led to the suitable (*R*)-**3** diol which showed an ee of 80%. This indicated that about 20% of enantiopurity was lost (i.e. that 10% of the wrong enantiomer was formed in the course of this step). Because of this last result, it was not possible to use directely the equations developped for the lipase/Mitsonobu process. Therefore, we carried out a similar mathematical development, corresponding to the theoretical scheme 3, which took into account the chemical inversion (x) at the benzylic carbon (eq. 1-4). The theoretical curves generated from equation (5) provide a useful overview of

the interrelationships between the enantiomeric excess of the residual epoxide (ee_s), reached just before chemical hydrolysis, and the enantiomeric excess of the final diol (ee_f) (after enzymatic and acid hydrolysis) for fixed values of E and x (Scheme 4). These graphical representations thus allow accurately to "tune" the acid hydrolysis in order to maximize ee_f once the values of E and x have been determined. It should be noted that the optimum ee_s values (ee_{sopt}) when ee_f is maximum (ee_{fmax}) can be easily obtained as a function of E (eq. 6) by calculating the zero point of the derivatized equation (5). In addition, substitution of equation (6) into equation (5) eliminates the ee_s and yields relationship between ee_{fmax}, E and x (eq. 7).

Scheme 3



$$(1)\quad eef = \frac{(Rd+R'd+R''d)-(Sd+S'd+S''d)}{(Rd+R'd+R''d)+(Sd+S'd+S''d)}$$

$$(2)\quad eef = \frac{Ro-So+2.x.(Se-Re)}{Ro+So}$$

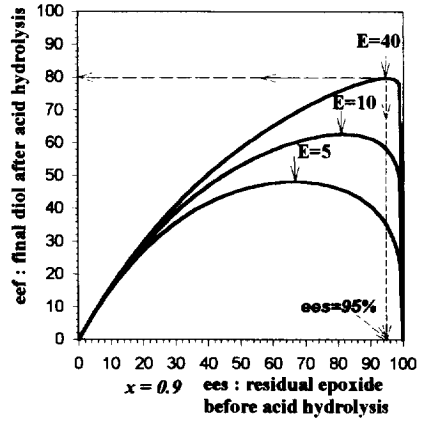
Ro=So

$$(3)\quad eef = 2.x.ee_S.(1-c)$$

Ro = So : enantiomeric composition of epoxide at t = 0
Re, Se : residual epoxide before acid hydrolysis
Rd, R'd, R"d : formed diol concentrations of (R) configuration
Sd, S'd, S"d : formed diol concentrations of (S) configuration

$$(4)\quad E = \frac{\ln[(1-c)(1-ee_S)]}{\ln[(1-c)(1+ee_S)]}$$

Scheme 4



$$(5)\quad ee_f = 2.x.ee_s.\left[\frac{(1+ee_s)^E}{(1-ee_s)}\right]^{\frac{1}{1-E}}$$

$$(6)\quad ee_{opt} = \frac{E-1}{E+1}$$

$$(7)\quad ee_{f\,max} = x.(E-1).E^{\frac{E}{1-E}}$$

Application of equation (6) to our process (for E = 40) leads to conclude that the acid hydrolysis (of the remaining epoxide **2**) must be performed when ee_s reaches a value of about 95%. From equation (5) or equation (7) we can determine that ee_f (for x = 0.9) should then be of about 80%.

Synthesis of (R)- Niténalol® 1.

Based on this mathematical approach, the combined enzymatic/acid catalyzed hydrolysis of racemic **2** was thus conducted on a preparative scale. The microbiological whole-cell step was thus followed until the ee of the remaining (*S*)-**2** epoxide reached the value of 95% (2.5 h), the vessel was then rapidly cooled to 10°C and a solution of sulfuric acid (18N) was added to the culture. This was stirred further for 16 h. After work-up, this led as expected to 90% yield of (*R*)-**3** which showed an ee of 83%. Recrystallization afforded 73% yield of optically pure material.

Because of the heterogeneity of such whole-cell cultures, and because the specific activity of whole-cell biocatalysts is intrinsically quite low as compared to the one of partly purified enzymatic extracts, an ultimate improvement of this synthesis could be devised based on our previous work. Indeed we have previously devised a method allowing the preparation of a water soluble enzymatic extract from *A. niger*[21] showing an enzymatic activity about 20 times higher than the one of the cultured mycelium (dry weight). This could be lyophilized and the obtained powder was shown to be stable for months when stored à +4°C without noticeable loss of activity (< 5%). This preparation could for instance be achieved starting from a 5 L fermentor jar culture, thus leading to a total of 400 mg of enzymatic powder which showed a total activity of 174 U/g towards epoxide **2**.

Using this water soluble extract, it was then possible to achieve the preparation of enantiopure (*R*)-**3** in homogeneous conditions. Thus, an amount of enzymatic extract corresponding to 27 U[22] was dissolved in 18.5 mL of a DMF/water (20/80) solution, and 1 g (330 mM) of racemic epoxide **2** (i.e. 53 g/L) was added. After 26 h, the remaining epoxide reached a 95% ee value, and an amount of 25 mL DMF was then added to stop the enzymatic hydrolysis and to dissolve completely all the residual epoxide. After cooling at -20°C, sulfuric acid (18N) was added and the medium was stirred for an additional 20 h period. After disappearence of the remaining epoxide and neutralisation, 1.04 g of (*R*)-diol **3** (94% yield) showing an ee of 80%, was isolated. This could be recrystallized to afford 0.81 g of enantiopure product.

At this stage of our synthesis, we used the above obtained (*R*)-**2** epoxide as enantiopure synthon to perform the synthesis of (*R*)-Niténalol® **1**. Thus, (*R*)-**3** could be recyclized into the corresponding (*R*)-**2** epoxide (89% yield) without loss of enantiopurity following a procedure similar to the one previously described by Golding et *al.*[23] (*R*)-**2** was further treated by a solution of isopropylamine in ethanol[24] leading (after 72 h at room temperature) to a totally regioselective attack at the less hindered carbon atom of the oxirane moiety which afforded enantiopure (*R*)-**1** in 90% yield.

CONCLUSION

Starting from racemic *para*-nitrostyrene oxide, we here describe the synthesis of the biologically active enantiomer of (*R*)-Nifénalol® **1**, a β-adrenergic blocker with antianginal and antiarhythmic properties. This method is based on a combined chemoenzymatic approach which allowed the preparation of (*R*)-**3** in an enantioconvergent manner. It implied the consecutive use of (a) an enantioselective hydrolysis of the epoxide moiety obtained by using either a whole-cell suspension or a soluble enzymatic extract of the fungus *Aspergillus niger* and (b) an acid catalyzed hydrolysis of the remaining epoxide. Improvement of this approach was devised by combining these two steps in a one-pot procedure and by optimizing the yield and ee of the diol produced by means of a mathematical approach. This allowed the preparation of enantiopure (*R*)-Nifénalol® **1** with a 58% overall yield following a four-step strategy including a resolution step. This type of methodology appears to be complementary to either the enantioconvergent enzymatic hydrolysis of racemic epoxides or the possible concomitant use of two enantio- and regiocomplementary biocatalysts which have been exemplified on some other targets.

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EXPERIMENTAL PART

General. The strain of *A. niger* used in this work is registered at the "Museum d'Histoire Naturelle" (Paris) under n° LCP 521 (Lab. de Cryptogamie, 12 rue Buffon, 75005 Paris, France). NMR spectra (^1H and ^{13}C) were recorded in $CDCl_3$ solution on a Bruker AC 250. Chemical shifts are reported in δ from TMS as internal standard. Optical rotation values were measured on a Perkin-Elmer 241C polarimeter at 589 nm. Determination of the enantiomeric excesses of epoxide **2** and diol **3** were performed using chiral columns as previously described.[18]

Biohydrolysis of *p*-nitrostyrene oxide 2 with whole cells of *A. niger*

The fungal strain was cultured in a 7 L fermentor as previously described.[14] After incubation for 43 h, the mycelium was filtered off, washed with water, and then transferred to a 2 L fermentor containing 1 L of pH 8 (0.1 M) phosphate buffer. The medium was stirred at 1100 rpm and maintained at 27°C. Racemic **2** (5 g) as a solution in DMF (50 mL) was added to the medium

Without acid hydrolysis: When the ee of **2** reached 95% (2 h) the reaction was stopped by adding CH_2Cl_2 (500 mL). The mycelium was filtered off and after decantation the aqueous phase was saturated with NaCl and then continuously extracted with CH_2Cl_2 (5 days). **2** and **3** were separated and purified from the crude product by flash chromatography (pentane/ether/MeOH). (*S*)-**2** (1.9 g, 38%) (ee 95%) was obtained as a pale yellow solid: mp 84°C; $[\alpha]^{25}_D$ = 36 (c 1.25, $CHCl_3$) and the (*R*)-diol **3** (2.7 g, 49%) (ee 66%) as a white solid: mp 78°C; $[\alpha]^{25}_D$ = -13.5 (c 0.92, MeOH). The ^1H and ^{13}C NMR data were identical to those described previously.[18]

With acid hydrolysis: When the ee of **2** reached 95% (2.5 h) the medium was quickly cooled to 10°C with an ice bath and cold H_2SO_4 (18 N, 111 mL) was added slowly in order to maintain the temperature at the same level. After 16 h mixing at low temperature, the mycelium was filtered off and the fungal cake washed with $CHCl_3$. The medium was neutralized with K_2CO_3 then continuously extracted with $CHCl_3$ (24 h). After standard work-up 5 g (90%) of (*R*)-**3** (ee 83%) were isolated.

Biohydrolysis of *p*-nitrostyrene oxide 2 with enzymatic extract from *A. niger*

175 mg (27 U) of enzymatic powder having a total activity of 154 U/g, obtained as previously described,[21] were dissolved by magnetic stirring in 15 mL of phosphate buffer (pH 8, 0.1 M) and the temperature was maintained at 25°C. After 5 min

of incubation 1 g of racemic epoxide **2** was added as a solution in DMF (3.5 mL). When the ee of the residual epoxide **2** exhibited a value of 95% (26 h) 25 mL of DMF were added to stop the enzymatic reaction and to dissolve all the residual epoxide. After cooling at -20°C, H_2SO_4 (18 N, 13.5 mL) was slowly added and the medium was mixed at -20°C until all the epoxide **2** had disappeared (20 h). 50 mL of water were added and the medium was neutralized with $NaHCO_3$ then saturated with sodium chloride. After extraction of the medium with AcOEt (100 mL, 3 times) the aqueous phase was continuously extracted with CH_2Cl_2 (24 h). Standard work-up of the combinated organic phases yielded 1.04 g (94%) of (*R*)-**3** (ee 80%). Recrystallisation of diol-**3** in $CHCl_3$ led to 0.81 g of optically pure (*R*)-**3**.

Synthesis of (*R*)-2 from (*R*)-3

0.65 g (89% yield) of enantiopure (*R*)-**2** was obtained by cyclisation of the corresponding acetoxy-bromide formed by reaction between 0.81 g of optically pure diol (*R*)-**3** and hydrogen bromide in acetic acid as previously described.[23]

Synthesis of (*R*)-Nifénalol®

1.77 g of isopropylamine was added to a stirred solution of 1 g of (*R*)-**2** in dry ethanol (10 mL). After 24 h all the epoxide was consumed and the excess of isopropylamine was removed under vacuum. A flash chromatography ($CHCl_3$/MeOH) of the crude product led to 1.2 g (90% yield) of (*R*)-Nifénalol®-**1** as an orange solid: mp 118-121°C. H^1 NMR δ: 1.08 (d, 3H, CH_3, J = 3.7 Hz); 1.10 (d, 3H, CH_3, J = 3.7 Hz); 2.60 (dd, 1H, H_2, J_{gem} = 12.2 Hz; J_{1-2} = 9.12 Hz); 2.91 (m, 1H, CH); 2.98 (dd, 1H, H_2, J_{1-2} = 3.6 Hz; J_{gem} = 12.2 Hz); 3.12 (s large, 1H, OH); 4.78 (dd, 1H, H_1, J_{1-2} = 3.6 Hz; J_{1-2} = 9.10 Hz); 7.55 (d, $2H_{arom}$, J = 8.6 Hz); 8.21 (d, $2H_{arom}$, J = 8.5 Hz). ^{13}C NMR δ: 22.8-23.0 ($2CH_3$); 48.9 (CH); 54.2 (CH2); 70.9 (CH); 123.6; 126.5; 147.3; 150 (C_{arom}). **1** was dissolved in dry ether and by bubbling HCl into the solution, the (-)-Nifénalol® hydrochloride was obtained as a white solid, mp: 208-211°C, lit.[17] 217-218°C. $[\alpha]^{20}_D$ = -40.3 (c 1.07, H_2O) lit.[17] $[\alpha]^{20}_D$ = -41 (c 2, H_2O).

References and Notes

1. Katsuki, T. *Coord. Chem. Rev.* **1995**, *140*, 189-214
2. Jacobsen, E.S.; Kakiuchi, F.; Fonsler, R.G.; Larrow, J.F.; Tokunaga, M. *Tetrahedron Lett.* **1997**, *38*, 773-776.
3. Kolb, H.C.; van Nieuwenhze, M.S.; Sharpless, K.B. *Chem. Rev.* **1994**, *94*, 2483-22547
4. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Bull. Chem. Soc. Fr.* **1995**, *132*, 769-800
5. Besse, P.; Veschambre, H. *Tetrahedron* **1994**, *50*, 8885-8927
6. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Tetrahedron* **1996**, *52*, 4593-4606
7. Bellucci, G.; Chiappe, C.; Cordoni, A. *Tetrahedron: Asymmetry* **1996**, *7*, 197-202
8. Wixtrom, R.N.; Hammock, B.D. In: *Biochemical pharmacology and toxicology*, **1985**, eds. D. Zakim, D.A. Vessey, 1:1-93. New York: Wiley
9. Tseng, H.F.; Laughlin, L.T; Lin, S; Armstrong, R.N. *J. Am. Chem. Soc.* **1996**, *118*, 9436-9437
10. Borhan, B.; Jones, A.D.; Pinot, F.; Grant, D.F.; Kurth, M.J.; Hammock, B.D. *J. Biol. Chem.* **1995**, *270*, 26923-26930
11. Blee, E.; Schuber, F. *Eur. J. Biochem.* **1995**, *230*, 229-234
12. Faber, K.; Mischitz, M.; Kroutil, W. *Acta Chem. Scand.* **1996**, *50*, 249-258
13. Chen, X.-J.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1993**, *58*, 5528-5532
14. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1993**, *58*, 5533-5536
15. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Bioorg. Med. Chem.* **1994**, *2*, 609-616
16. Murmann, W.; Rumore, G.; Gamba, A. *Bull. Chim. Farm.* **1967**, *106*, 251-268
17. Almirante, L.; Murmann, W. *J. Med. Chem.* **1966**, *9*, 650-653
18. Pedragosa-Moreau, S.; Morisseau, C.; Zylber, J.; Archelas, A; Baratti, J.C.; Furstoss, R. *J. Org. Chem.* **1996**, *61*, 7402-7407
19. Vänttinen, E.; Kanerva, L.T. *Tetrahedron: Asymmetry* **1995**, *6*, 1779-1786
20. Chen, C.S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1982**, *104*, 7294-7298
21. Morisseau, C.; Nellaiah, H.; Archelas, A.; Furstoss, R.; Baratti, J.C. *Enzyme Microb. Technol.* **1997**, *20*, 446-452
22. 1 enzymatic unit (U) allows to transform 1 µmole/min of substrate.
23. Golding, B.T.; Hall, D.R.; Sakrikar, S.J. *J. Chem. Soc., Perkin trans. 1* **1973**, *1*,1214-1220
24. Teotino, U.M.; Polo Friz, L.; Steis, G.; Della Bella, D. *Il Farmaco - Ed. Sc.* **1962**, *17*, 252-265

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