Tetrahedron Letters, Vol. 34, No. 30, pp. 4769-4772, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Aliphatic (*S*)-Cyanohydrins by Enzyme Catalyzed Synthesis

Norbert Klempier and Herfried Griengl*

Christian Doppler Laboratory for the Chemistry of Chiral Compounds at the
Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria

Marianne Hayn

Institute of Biochemistry, University of Graz, Schubertstraße 1, A-8010 Graz

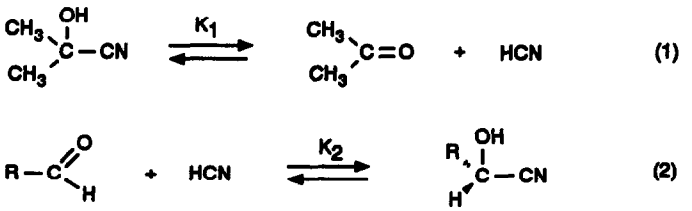
Abstract: **The enzyme catalyzed synthesis of aliphatic (*S*)-cyanohydrins has been accomplished for the first time by application of a hydroxynitrile lyase from leaves of *Hevea brasiliensis*. The optical purities of (*S*)-cyanohydrins obtained are in a range between 60 - 97%.**

Enantiopure cyanohydrins are valuable building blocks for chemical syntheses giving rise to important classes of compounds such as α-hydroxy acids, α-hydroxy ketones, ß-amino alcohols and pyrethroid pesticides. Therefore, considerable interest has been devoted to the development of preparative methods for these chiral synthons.[1-8] High enantiopurity is obtained by using kinetic resolution of racemic cyanohydrin derivatives catalyzed by lipases or esterases[9-13] as well as employing the enzyme catalyzed cyanohydrin reaction. Aromatic (*R*)- and (*S*)- cyanohydrins and aliphatic (*R*)-cyanohydrins are available by the latter method using either (*R*)-hydroxynitrile lyase from almonds (E.C.4.1.2.10)[14-23] or (*S*)-hydroxynitrile lyases from *Sorghum bicolor* (E.C.4.1.2.11)[24-26] or *Ximenia americana*.[27,28] The chemoenzymatic asymmetric synthesis of aliphatic (*S*)-cyanohydrins, however, has not yet been achieved.

Recently a hydroxynitrile lyase was isolated from the leaves of *Hevea brasiliensis*.[29] Contrary to the previously mentioned enzymes we found that the application of this enzyme to cyanohydrin reactions results in the formations of both aromatic and aliphatic (*S*)-α-hydroxynitriles in good enantiopurity.

In order to obtain good enantiomeric excess in cyanohydrins it is important to supress the competing non-enzymatic reaction. Following procedures already known this can be achieved by either using organic solvents[15-18,25] or by carrying out the reaction in aqueous medium at a sufficiently low pH value[24] as well as by keeping the concentration of cyanide ion at a low level throughout the reaction.

In our experiments the reaction was carried out in aqueous medium at low pH by using acetone cyanohydrin as cyanide source. Attempts to carry out the reaction in organic solvents (e.g. ethyl acetate, heptane, toluene) and mixtures thereof with low content of water were not successful. The enzyme catalyzed transhydrocyanation has recently been applied to the synthesis of (*R*)-cyanohydrins.[18,23]



Equilibria (1) and (2) are efficiently catalyzed by hydroxynitrile lyase from *Hevea brasiliensis*. At a pH value of 4.0 no significant chemical reaction (without enzyme) takes place.[30] The ratio of the equilibrium constants K_1 and K_2 is in favour of the formation of cyanohydrins **2**.

In typical experiments[31] a twofold molar excess of cyanide donor (acetone cyanohydrin) is used. When applying 100 units of enzyme[32] per mmol substrate conversions of about 80 - 100 % were achieved within two hours . The only exceptions found were those regarding the formation of cyanohydrins **2e** and **2h**; here only 35 % conversion was obtained: This was probably due to the low solubility of the corresponding aldehydes **1e** and **1h** in aqueous buffer. The enantiomeric excess of (*S*)-cyanohydrins obtained by the method of enzymatic transhydrocyanation is given in table 1.[33,34] In most cases the enantiomeric excess obtained is within the range of 80 to 94%, except for the reaction of pivalaldehyde (**1c**) and 3-phenoxy-benzaldehyde (**1h**) where also steric constraints of the active site might be responsible. Following the reaction conditions given here, no 1,4-addition product of compound **1f** could be observed.

Table 1: Enzyme catalyzed synthesis of cyanohydrins (S)-2a-h

compd. No.	R	%ee [34]	compd. No.	R	%ee [34]
a	$CH_3(CH_2)_2$	80	e	$CH_3(CH_2)_7$	85
b	$(CH_3)_2CH$	81	f	$CH_2{=}CH$	84
c	$(CH_3)_3C$	67	g	C_6H_5	94
d	$CH_3(CH_2)_4$	84	h	$3\text{-}(C_6H_5O)C_6H_4$	20

Acknowledgements:

The authors wish to express their cordial thanks to Prof. W. Steiner, Institute of Biotechnology, Graz University of Technology, for helpful discussions and also for supplying us with hevea leaves. We are also grateful to P. Pöchlauer, ÖMV-Chemie Linz, for his help in providing us with leaves.

References and Notes

1. Y. Kobayashi, H. Hayashi, K. Miyaji and S. Inoue, *Chem. Lett.* **1986**, 931.
2. H. Minamikawa, S. Hayakawa, T. Yamada, N. Iwasawa and K. Narasaka, *Bull. Chem. Soc. Jpn.***1988**, *61*, 4379.
3. J. D. Elliott, V. M. F. Choi and W. S. Johnson, *J. Org. Chem.* **1983**, *48*, 2295.
4. M. T. Reetz, F. Kunisch, and P. Heitmann, *Tetrahedron Lett.* **1986**, *27*, 4721.
5. W. R. Jackson, G. S. Jayatilake, B. W. Matthews and C. Wilshire, *Austr. J. Chem.* **1988**, *41*, 203.
6. H. Nitta, D. Yu, M. Kudo, A. Mori and S. Inoue, *J. Am. Chem. Soc.* **1992**, *114*, 7969.
7. K. Tanaka, A. Mori and S. Inoue, *J. Org. Chem.* **1990**, *55*, 181.
8. A. Mori,, Y. Ikeda, K. Kinoshita and S. Inoue, *Chem. Lett.* **1989**, 2119.
9. F. Effenberger, B. Gutterer, T. Ziegler, E. Eckhardt and R. Aichholz, *Liebigs Ann. Chem.* **1991**, 47.
10. H. Ohta, S. Hiraga, K. Miyamoto and G.-I. Tsuchihashi, *Agric. Biol. Chem.* **1988**, *52*, 3023.
11. M. Inagaki, J. Hiratake, T. Nishioka and J. Oda, *J. Am. Chem. Soc.* **1991**, *113*, 9360.
12. A. van Almsick, J. Buddrus, P. Hönicke-Schmidt, K. Laumen and M. P. Schneider, *J. Chem. Soc., Chem. Commun.* **1989**, 1391.
13. H. Ohta, Y. Kimura and Y. Sugano, *Tetrahedron Lett.* **1988**, 6957.
14. W. Becker, H. Freund, and E. Pfeil, *Angew. Chem.* 77, 1139 (1965); *Angew. Chem. Int. Ed. Engl.* **1965**, *4*, 1079.
15. F. Effenberger, T. Ziegler and S. Förster, *Angew. Chem.* **1987**, 99, 491; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 458.
16. J. Brussee, W. T. Loos, C. G. Kruse and A. van der Gen, *Tetrahedron* **1990**, *46*, 979.
17. E. Wehtje, P. Adlercreutz and B. Mattiasson, *Biotechnol. Bioeng.* **1990**, *36*, 39.
18. V. I. Ognyanov, V. K. Datcheva and K. S. Kyler, *J. Am. Chem. Soc.* **1991**, *113*, 6992.
19. L. Rosenthaler, *Biochem. Z.* **1908**, *14*, 238.
20. W. Becker and E. Pfeil, *Biochem. Z.* **1966**, *346*, 301.
21. M. Schuman Jorns, *Biochim. Biophys. Acta* **1980**, *613*, 203.
22. M. K. Seely, R. S. Criddle and E. E. Conn, *J. Biol. Chem.* **1966**, *241*, 4457.
23. T.T. Huuhtanen and L. Kanerva, Tetrahedron Lett. **1992**, 1223.
24. U. Niedermeyer and M. R. Kula, *Angew. Chem.* **1990**, *102*, 423; *Angew. Chem. Int. Ed. Engl.***1990**, *29*, 386.
25. F. Effenberger, B. Hörsch, S. Förster and T. Ziegler, *Tetrahedron Lett.* **1990**, 1249.
26. C. Bové and E. E. Conn, *J. Biol. Chem.* **1961**, *236*, 207.
27. G. W. Kuroki and E. E. Conn, Proc. Natl. Acad. Sci. USA **1989**, *86*, 6978.
28. C. G. Kruse, H. W. Geluk and G. J. M. Van Scharrenburg, *Chim. oggi*, **1992**, *10*, (1-2), 59.
29. D. Selmar, R. Lieberei, B. Biehl and E. E. Conn, *Physiologia Plantarum* **1989**, *75*, 97.
30. Stability of acetone cyanohydrin in aqueous solution strongly depends on the pH. At a pH-value of 4 dissociation into free cyanide and acetone occurs to a minute extent only. At the same pH-value the present (S)-hydroxynitrile lyase from *Hevea brasiliensis* still remains 20 % of their optimum catalytic activity. See ref. 35.

31. Procedure:

Preparation of cyanohydrins (*S*)-**2a-2f**

Example: 200 units of crude enzyme were suspended in 15 ml 0.1 M Na-citrate buffer (pH 4.0). To this 0.2 g (2mM) of hexanal and 0.34 g (4 mM) of acetone cyanohydrin were added and the reaction was shaken at r.t. for two hours.
After this time there was a 93% conversion to the cyanohydrin (monitored by gas chromatography). Methylene dichloride (20 ml) was added and the aqueous phase was extracted twice. After removal of the solvent under reduced pressure the crude product was purified on silica gel with petroleum ether/ethyl acetate (4/1) as eluent and subjected to kugelrohr distillation to give 0.19 g (75%) of (*S*)-**2d** as a colorless oil. $[\alpha]_D^{20}$ -12.1° (c 1.4, chloroform); ee 84%. Lit.[36] $[\alpha]_D^{20}$ -4.2° (chloroform); ee 26%.

Preparation of cyanohydrins (*S*)-**2g** and (*S*)-**2h**

Example: 200 units of crude enzyme were suspended in 15 ml 0.1 M Na-citrate buffer (pH 4.0). To this 0.21 g (2mM) of benzaldehyde and 0.34 g (4 mM) of acetone cyanohydrin were added and the well sealed flask was shaken for two hours at r.t.
The reaction was monitored by TLC until almost all benzaldehyde had disappeared. The reaction was extracted twice with methylene dichloride (20 ml) and the solvent was removed under reduced pressure. The crude product was further purified on silica gel with ethyl acetate/benzene/methylene dichloride (1/15/25) as eluent to give 0.21 g (79%) of (*S*)-**2g** as a slightly yellow oil. $[\alpha]_D^{20}$ -46.1° (c 2.1, chloroform); ee 94%.

32. The leaves were obtained from Dr. Isidore Gomes, Bangladesh Jute Research Institute, Dhaka, Bangladesh.
33. The absolute configurations of the product cyanohydrins **2a-h** were assigned by optical rotation measurement. In addition, for compound **2d** the configuration was assigned based on the elution order of its diastereomeric menthyl carbonates; t_R = 32.28 min (major (*S*)-enantiomer) t_R = 32.60 min (minor (*R*)-enantiomer).
34. The enantiomeric excess for compounds **2a-g** was determined by gas chromatography of their respective menthyl carbonates on a 25 m x 0.25 μm DB-1701 fused silica capillary column with nitrogen gas as carrier; The enantiomeric excess for compound **2h** was determined by liquid chromatography of its (-)-MTPA ester on LiChrosorb Si 60 with hexane/ethyl acetate (25/1) as eluent.
35. D. Selmar, F. J. P. Carvalho and E. E. Conn, *Analytical Biochemistry* **1987**, *166*, 208.
36. H. Gountzos, W. R. Jackson and K. Harrington, *Austr. J. Chem.* **1986**, *39*, 1135.

(Received in Germany 22 April 1993)