

ENZYME-CATALYZED SYNTHESIS OF (*R*)-KETONE-CYANOHYDRINS AND THEIR HYDROLYSIS
TO (*R*)- α -HYDROXY- α -METHYL-CARBOXYLIC ACIDS¹

Franz Effenberger*, Brigitte Hörsch^{2a}, Franz Weingart^{2b},
Thomas Ziegler and Stefan Kühner^{2c}

Institut für Organische Chemie, Universität Stuttgart
Pfaffenwaldring 55, 7000 Stuttgart 80, W.Germany

Summary: (*R*)-Ketone-cyanohydrins (*R*)-**2** are obtained with high enantioselectivity from aliphatic ketones **1** and HCN in organic solvents using (*R*)-oxynitrilase (EC 4.1.2.10) as catalyst. Acid catalyzed hydrolysis of the cyanohydrins (*R*)-**2** affords the corresponding (*R*)- α -hydroxy- α -methyl-carboxylic acids (*R*)-**3** without measurable racemization.

Very few optically active cyanohydrins, derived from ketones, are described in the literature. High diastereoselectivity was observed for the optically induced addition of HCN to 17-ketone steroids³ as well as for the addition of allyl trimethyl silane to optically active acyl cyanides⁴. Ohta et al⁵ have reported, in a series of papers, on the enantioselective saponification of racemic ketone cyanohydrins with yeast cells of *Pichia* *miso*, with only moderate chemical yields, however.

Recently, oxynitrilase-catalyzed enantioselective addition of hydrocyanic acid to aldehydes and ketones, respectively, in aqueous media was described in a patent⁶. The only ketone mentioned in this patent was 3-methyl-cyclohexanone which was converted to the corresponding cyanohydrin without determination of its optical purity⁶.

We have demonstrated⁷ that enantioselectivity may be increased significantly if the (*R*)- or (*S*)-oxynitrilase catalyzed addition of HCN to aldehydes is carried out in an organic solvent instead of water. We now report on the enantioselective addition of HCN to ketones **1**, also in organic solvents and employing (*R*)-oxynitrilase (EC 4.1.2.10) as catalyst. The (*R*)-cyanohydrins (*R*)-**2** are obtained with good chemical yields and in high optical purity⁸ (see Table 1).

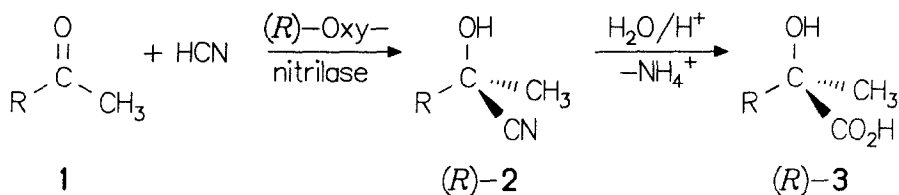


Table 1

(*R*)-Oxynitrilase Catalyzed Synthesis of (*R*)-Cyanohydrins (*R*)-2 and (*R*)- α -Hydroxy Carboxylic Acids (*R*)-3 from Ketones 1

1 R	react.cond.		(<i>R</i>)-2		(<i>R</i>)-3	
	time h	temp. °C	yield %	ee %	yield %	ee %
ethyl	41	0	80	76	85	76
n-propyl	43	0	70	97	64	99
n-butyl	42	0	90	98	72	98
n-pentyl	72	20	88	98	78	98
i-propyl	41	0	54	90	76	90
2-methylpropyl	40	0	57	98	89	96
3-methylbutyl	100	20	64	98	88	99
2-propenyl	16	0	68	94		
3-butenyl	24	20	80	97		
3-chloropropyl	120	0	87	84		

The enantiomeric purity of the ketone cyanohydrins (*R*)-2 cannot be determined from optical rotation. Since most of the data are not found in the literature, and since the optical rotation values are very small as a rule, an NMR spectroscopic determination with shift reagents of the respective ketone cyanohydrin acetates is not sufficiently precise⁵. We have therefore converted the cyanohydrins (*R*)-2 to diastereomeric esters with Mosher's Acid⁹, and determined the ee-values by gaschromatography of the diastereoisomers. For some cases, the cyanohydrins were acetylated and analyzed on a chiral cyclodextrin column¹⁰.

Optically active tertiary α -hydroxy acids are useful starting materials or synthetic intermediates for many chiral natural products, e.g. insect pheromones^{11a}, synthetic prostaglandin analogues^{11b}, α -tocopherol^{11c}. The synthesis of stereochemically pure tertiary acids is rather difficult and time consuming. From the many methods, described for the preparation of tertiary α -hydroxy acids¹², the alkylation of dioxolanes derived from chiral α -hydroxy carboxylic acids and pival aldehyde¹³ as well as the enantioselective hydrolysis of racemic α -methyl- α -benzyloxy carboxylates with a lipase¹⁴ yield acids with the highest optical purity.

We have obtained the important α -hydroxy- α -methyl carboxylic acids (R)-3 by simply hydrolysing the easily accessible ketone cyanohydrins (R)-2 with concentrated acid (see Table 1). The reaction sequence from the starting ketone 1 to the carboxylic acid (R)-3 can be carried out in one pot, without isolation of the cyanohydrins (R)-2¹⁵. Hydrolysis proceeds without measurable racemization as shown by comparison of the ee-values of the cyanohydrins (R)-2 and the hydroxy carboxylic acids (R)-3 (Table 1). With diazo methane the acids (R)-3 are converted to the corresponding methyl carboxylates and their optical purity is determined by gaschromatography on a chiral cyclodextrin column. The proof of the (R)-configuration of the cyano hydrins (R)-2 and the hydroxy acids (R)-3 was given by comparison of the optical rotation value of the (R)- α -hydroxy- α -methyl-butanoic acid (R)-3 (R = ethyl) with literature data¹⁶.

Acknowledgement: This work was generously supported by the Bundesministerium für Forschung und Technologie and the Fonds der Chemischen Industrie.

References

- 1) Enzyme-catalyzed Reactions, Part 9. - Part 8: F. Effenberger, B. Gutierrez, Th. Ziegler, Liebigs Ann.Chem., 1991,
- 2) a) B. Hörsch, Dissertation Univ. Stuttgart 1990. - b) F. Weingart, Forschungspraktikum Univ. Stuttgart 1989. - c) S. Kühner, Forschungspraktikum Univ. Stuttgart 1990.
- 3) a) A. Ercoli, P. de Ruggieri, J.Am.Chem.Soc. **75**, 650 (1953) - b) H. Kuhl, H.-D. Taubert, Steroids, **28**, 89 (1976). - c) D.A. Livingston, J.E. Petre, C.L. Bergh, J.Am.Chem.Soc. **112**, 6449 (1990)
- 4) M.T. Reetz, K. Kessler, A. Jung, Angew.Chem.Int.Ed.Engl. **24**, 989 (1985).
- 5) a) H. Ohta, Y. Kimura, Y. Sugano, Tetrahedron Lett. **29**, 6957 (1988) - b) H. Ohta, Y. Kimura, Y. Sugano, T. Sugai, Tetrahedron **45**, 5469 (1989)
- 6) U. Niedermeyer, U. Kragl, M.R. Kula, C. Wandrey, K. Makryaleas, K.H. Drauz, Europ.Pat.Ann.Nr. 0 326 063 A2 (23.01.89), Chem.Abstr. **112** (1990) 234 012 p.
- 7) a) F. Effenberger, Th. Ziegler, S. Förster, Angew.Chem.Int.Ed.Engl. **26** 458 (1987). - b) F. Effenberger, B. Hörsch, S. Förster, Th. Ziegler, Tetrahedron Lett. **31**, 1249 (1990).

- 8) (R)- α -Hydroxy- α -Methyl Alkane Nitriles 2: A solution of (R)-oxynitrilase (EC 4.1.2.10, 100 μ l, 1000 U/mol) was dropped on Avicel cellulose (1.5 g, soaked in 0.02 M sodium acetate buffer (pH 4.5)). Diisopropyl ether (20 ml) was added, followed by 5 mmol ketone 1 and 300 μ l hydrocyanic acid, and the reaction mixture was stirred at either 0°C or room temperature (Table 1). The catalyst was filtered off, washed with diisopropyl ether, and the combined filtrates were concentrated to give (R)-2. Example: From 0.57 g n-pentyl-methyl ketone, 0.62 g (R)- α -hydroxy- α -methyl heptane nitrile was obtained, $[\alpha]_D^{20} = +2.7^\circ$ (c = 1.12, chloroform).
- 9) J.A. Dale, D.L. Dull, H.S. Mosher, J.Org.Chem. **34**, 2534 (1969)
- 10) P. Fischer, R. Aichholz, U. Bölz, M. Juza, S. Krimmer, Angew.Chem.Int.Ed. Engl. **29**, 427 (1990).
- 11) a) K. Mori, Tetrahedron **45**, 3233 (1989). - b) J.S. Bindra "The Synthesis of Prostaglandins", in "The Total Synthesis of Natural Products"; John Wiley & Sons, N.Y. 1981, Vol. 4, p. 353. - c) T. Harada, T. Hayashiya, I. Wada, N. Iwa-ake, A. Oku, J.Am.Chem.Soc. **109**, 527 (1987) and references cited therein.
- 12) a) A.I. Meyers, J. Slade, J.Org.Chem. **45**, 2785 (1980) - b) X.C. He, E.L. Eliel, Tetrahedron **43**, 4979 (1987). - c) G. Boireau, A. Deberley, D. Abenheim, Tetrahedron **45**, 5837 (1989) - d) S.S. Jew, S. Terashima, K. Koga, Tetrahedron **35**, 2337 (1979)
- 13) D. Seebach, R. Naef, G. Calderari, Tetrahedron **40**, 1313 (1984)
- 14) T. Sugai, H. Kakeya, H. Ohta, J.Org.Chem. **55**, 4643 (1990)
- 15) (R)- α -Hydroxy- α -Methyl-Carboxylic Acids 3: A solution of the crude product (R)-2, prepared from 2 mmol ketone 1, as described in ref.8 was stirred in conc. HCl (5 ml) 16h at room temperature, followed by heating under reflux for 5h. The mixture was poured into water, extracted with diethyl ether, and the organic layers were dried. The residue, after filtration and concentration was crystallized from n-hexane. Example: From crude (R)- α -hydroxy- α -methyl heptane nitrile, prepared from 0.23 g, n-pentyl-methyl ketone, 0.25 g (R)- α -hydroxy- α -methyl-heptanoic acid was obtained, $[\alpha]_D^{20} = -9.4^\circ$ (c = 0.35 chloroform), m.p. 63°C (hexane) for the racemate the m.p. is 45°C; R.W. Stoughton, J.Am.Chem.Soc. **63** 2376 (1941).
- 16) (R)- α -Hydroxy- α -methyl-butanoic acid, $[\alpha]_D^{20} = -5^\circ$ (c = 0.34, chloroform); Ref. $[\alpha]_D^{20} = -8.5^\circ$ (c = 3, chloroform): B.W. Christensen, A. Kjaer, Acta Chem.Scand. **16**, 2466 (1962).

(Received in Germany 7 February 1991)