Tetrahedron Letters,Vol.29,No.52,pp 6957-6960,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

KINETIC RESOLUTION OF KETONE CYANOHYDRIN ACETATES WITH A MICROBIAL ENZYME

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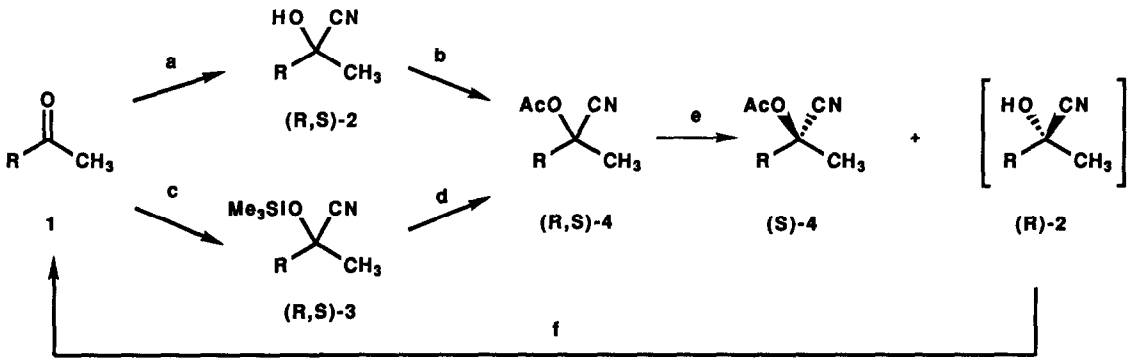
Summary: Incubation of dl-1-cyano-1-methylalkyl (or alkenyl) acetates (methyl ketone cyanohydrin acetates) with cells of Pichia miso IAM 4682 afforded optically active acetates and the corresponding ketones via asymmetric hydrolysis. Resulting (S)-2-cyano-2-undecyl acetate was converted to the aminofuranone derivative without losing its optical purity.

Cyanohydrins (α-cyanoalkanols) and related compounds have been used in organic synthesis via derivation to α-hydroxycarboxylic acid[1] and reactions with nucleophiles.[2] As they are useful in asymmetric synthesis[3] or esters of one mirror isomer of α-cyanoalkanols are known to be physiologically active,[4] preparation of optically active α-cyanoalkanols is considered to be important problem in organic synthesis. Concerning the synthesis of chiral 1-cyano-1-alkanols and their esters, some methods have been disclosed including biochemical[5] or catalytic[6] reactions and via diastereoselective reactions.[7] On the other hand, relatively few reports have been demonstrated so far on the preparation of ketone cyanohydrins.[8] As these compounds are considered to be an interesting class of chiral building blocks, we tried to obtain them via microbial kinetic resolution of the corresponding acetates.

We have already reported that esterases of *Bacillus coagulans* and *Candida tropicalis* are effective to kinetic resolution of a number of carboxylic esters of 1-cyano-1-alkanols.[9] Thus, at first we applied these microorganisms to the kinetic resolution of 1-cyano-1-methyldecyl acetate (**4d**) only to get disappointing results. Then, type cultures of our laboratory have been screened. Among them, *Pichia miso* IAM 4682 (a kind of yeast) has been found to be the best for the distinction of the chirality of **4d**. Representative experimental procedures are as follows. *Pichia miso* was grown in 50 ml of a medium consisted of glucose (1%), yeast extract (0.5%), peptone (0.7%) and K_2HPO_4 (0.5%) for 2 days at 30 °C. The pH of the medium was initially adjusted to 7.2. The substrate **4d** was added to the suspension of grown cells at a concentration of 0.3% to the medium, and the incubation was continued for additional 2 days. The broth was extracted with ethyl acetate (50 ml x 3), and the organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a mixture of unreacted acetate and the corresponding ketone resulting via decomposition of hydrolyzed 2-cyano-2-undecanol (**2d**). Preparative TLC repeated

twice on silica gel with hexane-ethyl acetate (10:1) gave pure 1-cyano-1-methyldecyl acetate in 38% yield and 2-undecanone. The acetate showed a specific rotation of $[\alpha]_D^{31}$ -16.7° (c 1.49, benzene). The optical purity was over 95%, based on ^{1}H-NMR spectrum obtained in the presence of Eu(tfc)$_3$.[10]

Present enzymatic hydrolysis was applied to various α-cyanoalkyl and alkenyl acetates. The results are summarized in Table 1. In all cases hydrolyzed cyanohydrins were recovered as the corresponding ketones with liberation of hydrogen cyanide. As the ketones are the starting materials for the preparation of dl-cyanohydrin acetates, recycling of the resulting ketones enables to convert the whole racemic acetate to a single enantiomer without a racemization process in principle. 1-Cyano-1-methylpropyl acetate (**4a**) was hydrolyzed



a. 1) $Na_2S_2O_5$ 2) KCN b. Ac_2O/Py c. Me_3SiCN d. Ac_2O/$FeCl_3$ e. *Pichia miso*

f. Decomposition under Cultivation Conditions

Table 1. Microbial Hydrolysis of Ketone Cyanohydrin Acetates

	R	Conc (%)	Cult (day)	Recovery (%)	$[\alpha]_D$ (°)	e.e. (%)
a	C_2H_5	0.3	0.5	25	-	0
b	C_3H_7	0.3	0.5	28	-15.0	>95[a]
c	C_6H_{13}	0.3	2	38	-19.7	>95[a]
d	C_9H_{19}	0.3	2	39	-16.7	>95[a]
e	$(CH_3)_2CH$	0.3	0.5	35	+3.9	9[a]
f	CH_2=CHC_3H_6-	0.3	2	32	-24.5	>99[b]
g	Ph	0.2	4	37	-	0[c]
h	$PhCH_2$	0.2	5	45	-	0[a]

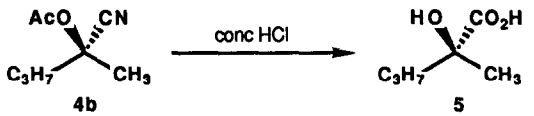
[a]Determined by ^{1}H-NMR (90 MHz) in the presence of Eu(tfc)$_3$.

[b]Determined by ^{1}H-NMR (400 MHz) in the presence of Eu(tfc)$_3$.

[c]Determined by HPLC.

smoothly, but the recovered acetate was racemic. The enzyme of *P. miso* seems to be unable to distinguish an ethyl from a methyl group. On the other hand a propyl group is long enough to be recognized as a different radical from a methyl. 1-Cyano-1-methylalkyl acetates with longer alkyl chains were also hydrolyzed with high enantioselectivity, although the reaction proceeded rather slowly. An olefinic bond had no negative effect to the reaction (**4f**). In contrast to these substrates, acetates with branched alkyl group was hydrolyzed non-selectively. Aromatic compounds are no good substrates, neither.

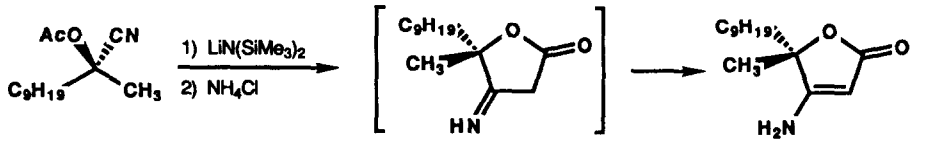
The absolute configurations of the recovered acetates were estimated as follows. Optically active 1-cyano-1-methylbutyl acetate (**4b**) was heated under reflux overnight in conc. hydrochloric acid. Extraction with diethyl ether and purification with preparative TLC on silica gel afforded optically active 2-hydroxy-2-methylpentanoic acid (**5**) in ca. 40% yield,[11] which exhibited the



4b **5**

$[\alpha]_D^{25}$ +9.53° (c 2.12, $CHCl_3$). As the specific rotation of (R)-2-hydroxy-2-methylbutanoic acid has been reported to be $[\alpha]_D^{25}$ -8.5° (c 3, $CHCl_3$),[12] the absolute configuration of **5** was suggested to be S. In addition, comparison of ^{1}H-NMR peaks due to acetyl group of optically active **4b, c, d, f** with those of racemic compounds in the presence of $Eu(tfc)_3$, it is concluded that the peak in the higher field always disappeared in the spectra of chiral acetates. This fact strongly suggests that the configuration is identical between these optically active acetates. Only in the case of **4e**, which has an opposite optical rotation to other acetates, the peak in the higher field was the stronger of the 2 peaks due to acetyls of 2 enantiomers. Thus, the absolute configuration of recovered acetates in the case of **4b, c, d, f** were estimated to be S.

Optically active acetates now available by the microbial hydrolysis can be applied to the synthesis of optically active 4-amino-2(5H)-furanones, which have been known to have interesting physiological activities, according to the procedures developed by Hiyama *et al.*[13] As an example, chiral **4d** was converted to aminofuranone **7**. Optically active acetate **4d** was treated in THF with excess lithium hexamethyldisilazide at -78 °C for an hour. Evaporation of the solvent after quenching the reaction mixture with aqueous NH_4Cl, followed by purification of the residue with column chromatography afforded aminofuranone **7** as colorless crystals in a yield of 70%.[14] Mp. 106-107 °C, $[\alpha]_D^{22}$ -4.4° (c 1.63, $CHCl_3$). HPLC analysis[15] revealed that the optical purity of resulting **7** is over 95%, which indicates the base-catalyzed cyclization process is accom-



4d **6** **7**

panied by no racemization.

Acknowledgement: The authors are greatful to Dr. Tamejiro Hiyama of Sagami Chemical Research Center for his helpful discussion.

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10) Addition of 0.5 eq. of the chiral shift reagent to the solution of dl-**4d** in CCl_4 resulted in the splitting of the singlet due to acetyl group by about 0.3 ppm. On the other hand, only a single peak was observed for the optically active acetate. When 5% of dl-**4d** was added to this solution the peak due to the another enantiomer was clearly detected.
11) The hydroxy acid was identified by spectroscopic measurements; ^{1}H-NMR ($CDCl_3$): δ 0.89 (t, 3H, J=6.6, CH_3), 1.42 (s, 3H, CH_3), 1.35-1.84 (m, 4H, CH_2CH_2), 6.87 (bs, 2H, OH). IR (neat) ν_{max} 3450, 2960, 1710, 1450, 1230, 1150, 1050 cm^{-1}.
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14) ^{1}H-NMR ($CDCl_3$): δ 0.77-1.23 (m, 17H, C_8H_{17}), 1.44 (s, 3H, CH_3), 1.57-1.85 (m, 2H, CH_2), 4.73 (s, 1H), 4.82 (bs, 2H, NH_2). IR (KBr): ν_{max} 3450, 3050, 2950, 2850, 1735, 1710, 1660, 1580 cm^{-1}.
15) Column, DICEL Chiral Cel OA, 25 cm; Solvent, hexane-isopropanol (750:16); Flow rate, 0.5 ml/min; Retention time, 294 and 341 min.

(Received in Japan 11 October 1988)