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Silicon Effect on the Enantioselective Transcyanation of Acetyltrimethylsilane Examined by Different Oxynitrilases

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Abstract: The synthesis of optically active (R)- and (S)-2-trimethylsilyl-2-hydroxyl propionitrile by enantioselective transcyanation of acetyltrimethylsilane with acetone cyanohydrin was successfully carried out using defatted plum, loquat , peach, almond or apple seed meals as (R)-oxynitrilase source and using *Manihot esculenta* leaves as (S)-oxynitrilase source in a biphasic system with good conversion and high enantiomeric excess. Comparative study demonstrated that silicon atom in substrate showed great effect on the reaction and due to the unique characteristics of silicon atom, both the substrate conversion and the product *e.e.* of the transcyanation of acetyltrimethylsilane were much higher than those of its carbon counterpart 3,3-dimethyl-2-butanone for all examined oxynitrilases.

Keywords: Enantioselective transcyanation, cyanohydrin, oxynitrilase, acetyltrimethylsilane.

Over the past years, optically active cyanohydrins have earned much attention for their great importance in organic synthesis[1]. Oxynitrilases are a group of efficient biocatalysts for the preparation of optically active cyanohydrins by catalyzing the addition of hydrogen cyanide to carbonyl compounds[2].

Organosilicon compounds show particular chemical and physical properties compared with their carbon analogues owing to the unique characteristics of silicon atom. They could be used for the synthesis of drugs with better pharmacological effect, higher selectivity, and lower toxicity than their carbon counterparts, though little has been known about the mechanisms[3,4]. The replacement of specific carbon atoms in drugs by silicon seems to be a useful and efficient strategy in drug design and should be regarded as a complementary tool for the development of new drugs[5]. Recently, some investigations have been carried out in order to test the synthetic potential of biotransformation to produce useful organosilicon compounds, and to study in detail the mechanism of enzymatic catalysis as well[6]. We have already reported the silicon effect on dehydrogenase-catalyzed reaction, where the silicon atom served as a more effective atom than the carbon atom to enhance the activity and enantioselectivity of the compound[7].

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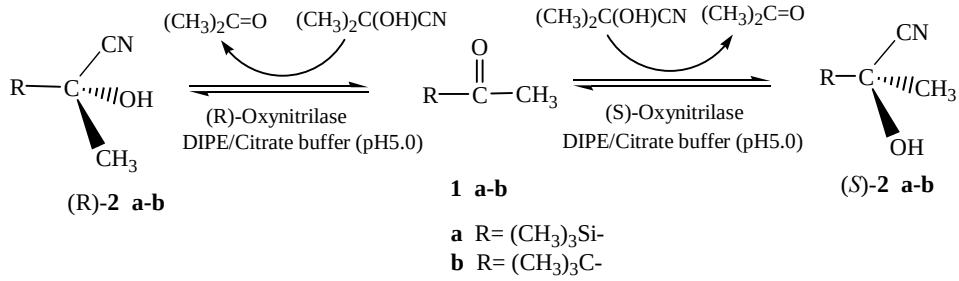
Scheme 1 Enantioselective transcyanation of acetyltrimethylsilane and its carbon counterpart



Synthesis of optically active (R)- and (S)-2-trimethylsilyl-2-hydroxyl propionitrile **2** by enantioselective transcyanation of acetyltrimethylsilane **1a** with acetone cyanohydrin (donor of HCN) was successfully carried out using plum, loquat, peach, almond or apple seed meals as (R)-oxynitrilase source and using *Manihot esculenta* leaves as (S)-oxynitrilase source in an aqueous/organic biphasic system. For the first time, comparative study with its carbon counterpart **1b** was systematically made by the oxynitrilases mentioned above to examine the silicon effect on the reaction (**Scheme 1**).

Experimental

Peach, plum, almond seeds were purchased from a local medical store, and the seeds of loquat and apple were collected from the fresh fruits. After being granulated, the preparations were defatted with ethyl acetate and stored at 4 °C for use. Fresh *Manihot esculenta* leaves were squeezed with a juice extractor. After deposition with $(NH_4)_2SO_4$, the proteins were collected by centrifugation at 8000×g for 30 minutes and the crude enzyme powders were obtained after lyophilization and stored at 4 °C.

n-Decane, acetyltrimethylsilane were purchased from Sigma and Aldrich (USA). Acetone cyanohydrin was from Tokyo Kasei Kogyo Co., Ltd (Japan). All other chemicals were from commercial sources and of analytical grade.

The reactions were carried out in a biphasic system. In a typical experiment, diisopropyl ether (10 mL) containing 14 mmol•L^{-1} acetyltrimethylsilane, 28 mmol•L^{-1} acetone cyanohydrin and 10 mmol•L^{-1} *n*-decane (used as internal standard) was mixed with citrate buffer (1.5 mL, 0.1 mol/L, pH 5.0) containing the enzyme preparation(500 mg). The mixture was incubated within a flask (50 mL) capped with a septum and shaken in a water bath shaker at 190 rpm.

Samples (0.2 mL) were taken from the organic phase at specific time intervals (more than 99% of the substrate was present in the organic phase and no side product was detectable by GC) and assayed by HP 4890 gas chromatography with a flame ionization detector and a chiral column (20% permethylated *β*-cyclodextrin 30 m × 0.25 mm × 0.25 μm, HP, USA). The column temperature was maintainined at 80 °C for 2 min and then being upgraded to 125 °C at the rate of 5 °C/min. The retention-times of acetyltrimethylsilane, acetone cyanohydrin, *n*-decane, (S)-product, and (R)-product were 1.722 min, 3.662 min, 5.548 min, 9.737 min and 9.843 min, respectively.

Results and Discussion

The defatted plum, loquat, peach, apple or almond seed meals were selected as (R)-oxynitrilase source and *Manihot esculenta* leaves as (S)-oxynitrilase source for the synthesis of **(**R**)-2a** and **(**S**)-2a** respectively by transcyanation of acetyltrimethylsilane (**1a**) with acetone cyanohydrin in the biphasic system of 13%(V/V) of citrate buffer (Ph 5.0) and diisopropyl ether. As can be seen in **Table 1**, acetyltrimethylsilane (**1a)** was a good substrate for most of the (R)-oxynitrilases explored. Acetyltrimethylsilane **(1a)** was transferred to **(**R**)-2a** with excellent substrate conversion (99%) and product *e.e.* (99%) using plum, apple, almond or peach seed meal, with good substrate conversion (98%) and product *e.e.* (92%) using loquat seed meal. In case with *Manihot esculenta,* acetyltrimethylsilane (**1a)** was transfered to **(**S**)-2a** with 99% conversion and 93% *e.e.*.

To examine the silicon effect on the reaction, comparative study was made with its carbon counterpart 3,3-dimethyl-2-butanone **(1b)**. As shown in **Table 1**, both the substrate conversion and the product *e.e.* of the transcyanation of **1a** were much higher than those of its carbon counterpart **1b** for all examined oxynitrilases. Especially in the case of loquat enzyme, **1a** was transferred to (R)-**2a** with good substrate conversion and product *e.e.*, but if the silicon atom in **1a** was substituted by corresponding carbon atom, aliphatic **1b** could not be accepted by loquat enzyme, as was reported that the loquat enzyme did not accept any aliphatic aldehydes or ketones[8]. These results demonstrated that the silicon atom in substrate served as a more effective atom than the carbon atom to enhance the activity and enantioselectivity of the enzymes, which is in agreement with our previous study about the effect of silicon atom in substrates on dehydrogenase-catalyzed reaction[7].

The markedly higher reactivity and enantioselectivity of oxynitrilase-catalyzed transcyanation of **1a** can be explained in terms of the structure and catalytic mechanism of oxynitrilase and the particular properties of silicon atom. Enzymatic kinetics established that the oxynitrlase-catalyzed reaction follows an ordered uni-bi mechanism with the carbonyl group binding to the active site of the enzyme followed by HCN attack on the complex[9]. Currently, the crystal structure for oxynitrilase from *Hevea brasiliensis* demonstrated that the active site of oxynitrilase buries deep inside the protein and connects to the surface by a narrow channel[10]. Therefore, the formation of the complex is greatly influenced by the size of the group linking to the carbonyl carbon atom. It has been also reported that the ability of a carbonyl compound to act as a substrate for the almond oxynitrilase decreased with increasing size of the group linking to the carbonyl carbon atom[11]. Similarly, due to the great steric hindrance of the group $(CH_3)_3C$-, the transcyanation of 3,3-dimethyl-2-butanone became much difficult. The silicon atom has a much longer covalent radius than the carbon atom, which gives larger space between the group $(CH_3)_3Si$- and carbonyl carbon atom and is propitious to the formation of the complex and the attack of HCN on the complex, resulting in higher reactivity. On the other hand, for oxynitrilase-catalyzed transcyanation, a spontaneous non-enzymatic chemical addition which forms the corresponding racemic cyanohydrin always competes with the highly enantioselective enzymatic reaction[8], and thus the increase in enzymatic reactivity may lead to an increase in the enantiomeric purity of the product.

Table1 Silicon effect on the enantioselective transcyanation examined by different oxynitrilases

Substrate	Source of enzyme	R/S selectivity	t(h)	Conversion(%)	*e.e.* (%)
$(CH_3)_3SiCOCH_3$(**1a**)	Plum	R	90	99	99
	Apple	R	24	99	99
	Almond	R	35	99	99
	Loquat	R	96	98	92
	Peach	R	24	99	99
	Manihot esculenta	S	48	99	93
$(CH_3)_3CCOCH_3$(**1b**)	Plum	R	150	16	38
	Apple	R	60	16	48
	Almond	R	50	17	81
	Loquat	R	72	0	0
	Peach	R	54	17	80
	Manihot esculenta	S	48	26	15

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