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Note

Cyclosophorohexadecaose and succinoglycan monomers as catalytic carbohydrates for the Strecker reaction

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Abstract—Some microbial carbohydrates have been used as catalysts for the multicomponent Strecker reaction using trimethylsilyl cyanide (TMSCN). α-Cyclosophorohexadecaose (α-C16) derived from *Xanthomonas* species and succinoglycan monomers derived from *Rhizobium* species acted as catalytic carbohydrates in the mixture solutions of methanol and water. Malonaldehyde bis(phenylimine) as a substrate was completely converted (yield: 100%) into its product to 100% by both α-C16 and the succinoglycan monomer (M2), having acetyl, pyruvyl, and succinyl groups as substituents after 1 h. The catalytic abilities of the carbohydrates were dependent on the inherent structures of the substrates used in this study, where substrate **1** having a symmetrical structure rather than the others was favorably reacted with the α-C16 and M2. Through this study, we suggest that the microbial carbohydrates used in this study could be expected to be environmentally-benign catalysts for the synthesis of α-aminonitriles.

Keywords: Catalytic carbohydrate; Microbial carbohydrate; α-Cyclosophorohexadecaose; Succinoglycan monomer; Strecker reaction

Cell-associated carbohydrates are known to be involved in bacterium–plant interactions in both pathogenesis and symbiosis.[1] In addition to the biological functions, several studies on biotechnological application using *Rhizobial* or *Bradyrhizobial* cyclic glucan have been recently reported.[2]

We have recently reported that *Rhizobial* cyclic β-(1,2)-D-glucans,[3] succinoglycan,[4] and *Zoogloeal* zooglan[5] as linear acidic polysaccharides, function as novel catalysts for the methanolysis of ester compounds. Based on the functions of these natural microbial carbohydrates, we investigated catalytic abilities of microbial cyclic or linear carbohydrates for the Strecker reaction in aqueous solution.

The Strecker reaction is one of the most practical and efficient methods for the synthesis of α-aminonitriles,[6] which are very useful precursors for the synthesis of α-amino acids, various nitrogen-containing heterocycles and other biologically useful molecules.[7] The Strecker reaction is generally carried out by the nucleophilic addition of cyanide ion to the imines using acid or base catalysts. The efficiency of the reaction has been increased by the use of catalyst, and reactive cyanide ion sources, which are hydrogen cyanide, sodium or potassium cyanide, bis(dialkylamino)cyanoboranes, diethylphosphorocyanidate, and trimethylsilyl cyanide (TMSCN). Among them, TMSCN is known to be easy to handle and more effective cyanide ion source for the nucleophilic addition reactions under mild conditions as compared to HCN, NaCN, or KCN. Homogeneous and heterogeneous catalysts have been used for the synthesis of α-aminonitriles.[7,8] However, most of these methods have one or more disadvantages such as expensive reagents, harsh conditions, and the generation of a large amount of toxic materials. Because of these reasons, clean and environmentally friendly solvent-free processes have been recently developed to afford α-aminonitriles in quantitative yields.[8]

In this study, we show that both α-cyclosophorohexadecaose (α-C16) derived from *Xanthomonas oryzae* and the succinoglycan monomer derived from *Rhizobium meliloti* function as environmentally-benign catalysts

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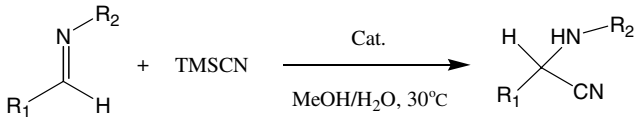

$R_1 = C_6H_5\text{-}N{=}CH\text{-}CH_2$, $R_2 = C_6H_5$: **1**
$R_1 = C_6H_5$, $R_2 = C_6H_5\text{-}SO_2$: **2**
$R_1 = C_6H_5$, $R_2 = (C_6H_5)_2\text{-}CH$: **3**

Figure 1. Scheme of the Strecker synthesis of α-aminonitriles catalyzed by microbial cyclosophorohexadecaose (α-C16) or succinoglycan monomers.

for the Strecker reaction (Fig. 1). α-C16 is a neutral cyclic hexadecaglucoside containing 15 β-(1→2)-linkages and 1 α-(1→6)-linkage and a periplasmic glucan isolated from rice pathogenic bacteria, *Xanthomonas oryzae* pv. *oryzae*.[9] On the other hand, succinoglycan monomers (M1–M3) are octasaccharides derived from *R. meliloti* 1021, which have acetyl, pyruvyl, and succinyl groups as substituents (Fig. 2).[10]

Structural analyses of the purified α-C16 were carried out through NMR spectroscopy and MALDI-TOF mass spectrometer and the results were the same as the previous reports.[9,11] Figure 2a shows the proposed structure of α-C16. A detailed structural characterization of the purified succinoglycan monomers was also carried out by NMR spectroscopy and the result was identical with the previous report.[10] From the structural information of the succinoglycan monomers, we have confirmed that the acetyl group is located at the C-6 position of the third glucose residue from the reducing terminus, the succinyl group is located at the C-6 position of the 6th and 7th glucose residue and the pyruvyl

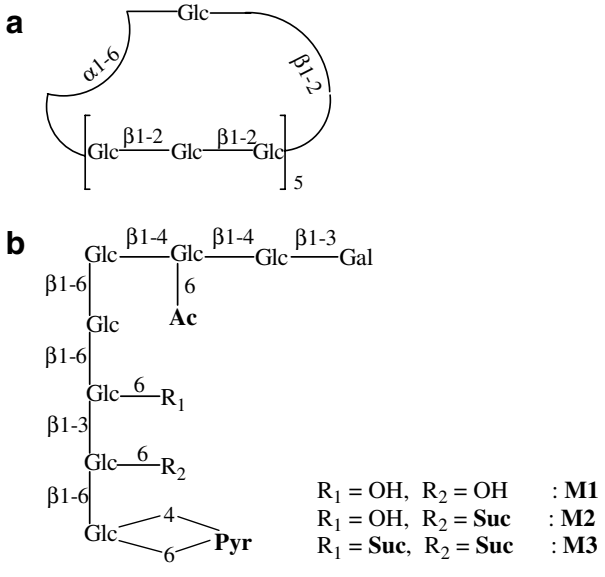

Figure 2. The proposed structures of (a) α-C16[9b,11] and (b) the succinoglycan monomers (Glc: glucose; Gal: galactose; Ac: acetyl; Suc: succinyl; Pyr: pyruvyl group).[10]

group is linked to the 8th glucose residue through a 4,6-ketal linkage. As shown in Figure 2b, succinoglycan monomers are composed of M1–M3, depending on their substituents.[10]

The finally purified α-C16 and succinoglycan monomers were used as catalysts for the Strecker reaction in the mixture solution of water and methanol. When α-C16 and M2 of succinoglycan monomers are added in the reaction media, the substrate **1** was converted into their products within 1 h in the mixture solutions of methanol and water and then the conversion was 100%. In this study, we determined the quantitative yields by ^{1}H NMR spectroscopic analysis. As shown in Figure 3, the proton d linked to the imine carbon corresponding to the reactant disappeared after 5 min and the proton d′ of the product clearly appeared in the presence of α-C16 (Fig. 3a) and M2 (Fig. 3b). For 30 min of the reactions, the signals corresponding to the ring protons (a–c) of **1** were shown with their product signals (a′–c′). Based on the structural symmetry of **1**, these results indicate that the cyanide ion as a nucleophile does not simultaneously attack the electrophilic imine carbons of **1**. Furthermore, the reaction patterns were different up to 30 min, depending on the added catalysts. In the presence of α-C16, the reactant still remained above 40% up to 30 min and finally the reaction was completed after 1 h (Fig. 3a). On the other hand, the reactant was converted into its product above 95% in the presence of M2 at 30 min (Fig. 3b). These results might be due to weak acidity of M2 having succinyl group as a substituent. UV–vis spectroscopic analysis was also performed with **1** alone and the mixture of α-C16 or M2 in the mixture of water and methanol after 30 min of reaction. The substrate **1** alone has a characteristic absorption around 380 nm in the mixture solution. Once the reaction is preceded by the two catalysts, the absorptions are gradually decreased around 380 nm, accompanied by a colorimetric change from yellow to colorless as shown in Figure 3c. From the UV–vis spectra, M2 was more effective than α-C16 during the reaction for 30 min. In the absence of α-C16 or M2, the conversion of **1** alone into its product and those obtained by adding β-cyclodextrin (β-CD) were below 10% even after 24 h, which were confirmed by ^{1}H NMR spectroscopic analysis (data not shown). In the case of **2**, among the succinoglycan monomers, the M1 and M3 converted the substrate into their products in 48% and 40% yield, respectively. In the case of **3**, the M1 and M3 converted the substrate into their products in 17% and 15% yield, respectively. α-C16 had no catalytic effect on the substrate **2** and **3** (Table 1). Through this study, we also investigated the enantiomer excess (ee) induced by the catalytic carbohydrates with a chiral column but all the ee ratios were below 10%. We also tested the effect of other microbial cyclic carbohydrates such as *Rhizobial* cyclic β-(1,2)-glucan (cyclosophora-

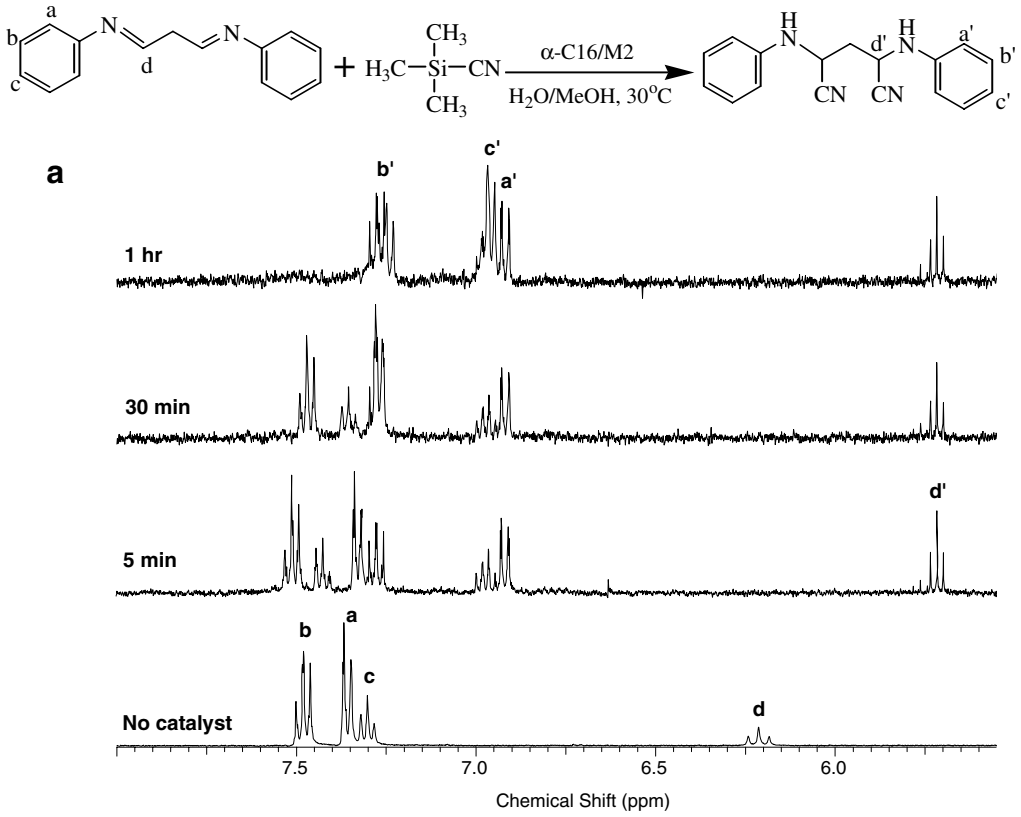

Figure 3. Partial ^{1}H NMR spectra of **1** in the absence or presence of (a) α-C16 and (b) the succinoglycan, M2, as catalyst at 0, 5, 30, and 60 min and (c) UV–vis absorption spectra of **1** (——) alone and the reaction mixture with α-C16 (--------) or M2 (—·—) after the reaction for 30 min.

ose) isolated from *Rhizobium* species and cyclic β-(1,3), β-(1,6)-glucan isolated from *Bradyrhizobium* species in the reaction but the two cyclic glucans have no effects on the reaction. Recently, it has been reported that β-CD acted as an effective catalyst for the Strecker reaction in water.[8b] However, β-CD did not show distinct catalytic effect for the imine compounds used in this study, in the mixture solution of water and methanol.

In addition to substrates above, *N*-benzylideneaniline, *N*-benzylidenebenzylamine, *N*-benzylidene-*tert*-butylamine and *N*-benzylidene-methylamine were also investigated for the Strecker reaction but no reaction occurred. These results indicated that α-C16 and the succinoglycan monomers participated in the Strecker reaction with regioselectivity toward the imine compounds, based on the most accessible reactivity shown toward the substrate **1** having a symmetric structure rather than the others.

From this study, we postulate that the possible mechanism of the Strecker reaction could be primarily due to hydrogen bondings of hydroxyl groups of the microbial catalytic cyclic or linear oligosaccharides with the nitrogen atoms of the imine compounds and then the imine carbon could be more electrophilic, activating it for nucleophilic attacking of the cyanide ions. This phenomenon strongly occurred in the reaction with substrate **1** rather than with the others. In addition to the hydrogen bonding effects, the structural properties of the α-C16 or the succinoglycan monomers might contribute to the catalytic reaction, depending on the substrates. Especially, the α-C16 could have complex-forming ability toward substrate **1**, based on its cyclic structure. Also, the succinoglycan monomers could act as general weak acids due to the anionic substituents within the structures.

In this study, we show for the first time that the microbial cyclic or linear glucans can function as catalytic carbohydrates for the Strecker reaction. From this study, we suggest that the microbial catalytic carbohydrates can be further applied as an environmentally-benign catalyst in various organic syntheses reactions. Further applicative experiments are in progress.

1. Experimental

1.1. General methods

All the substrates used in this study were purchased from commercial sources (Sigma or Aldrich Chem. Co.) and used without further purification. Water and

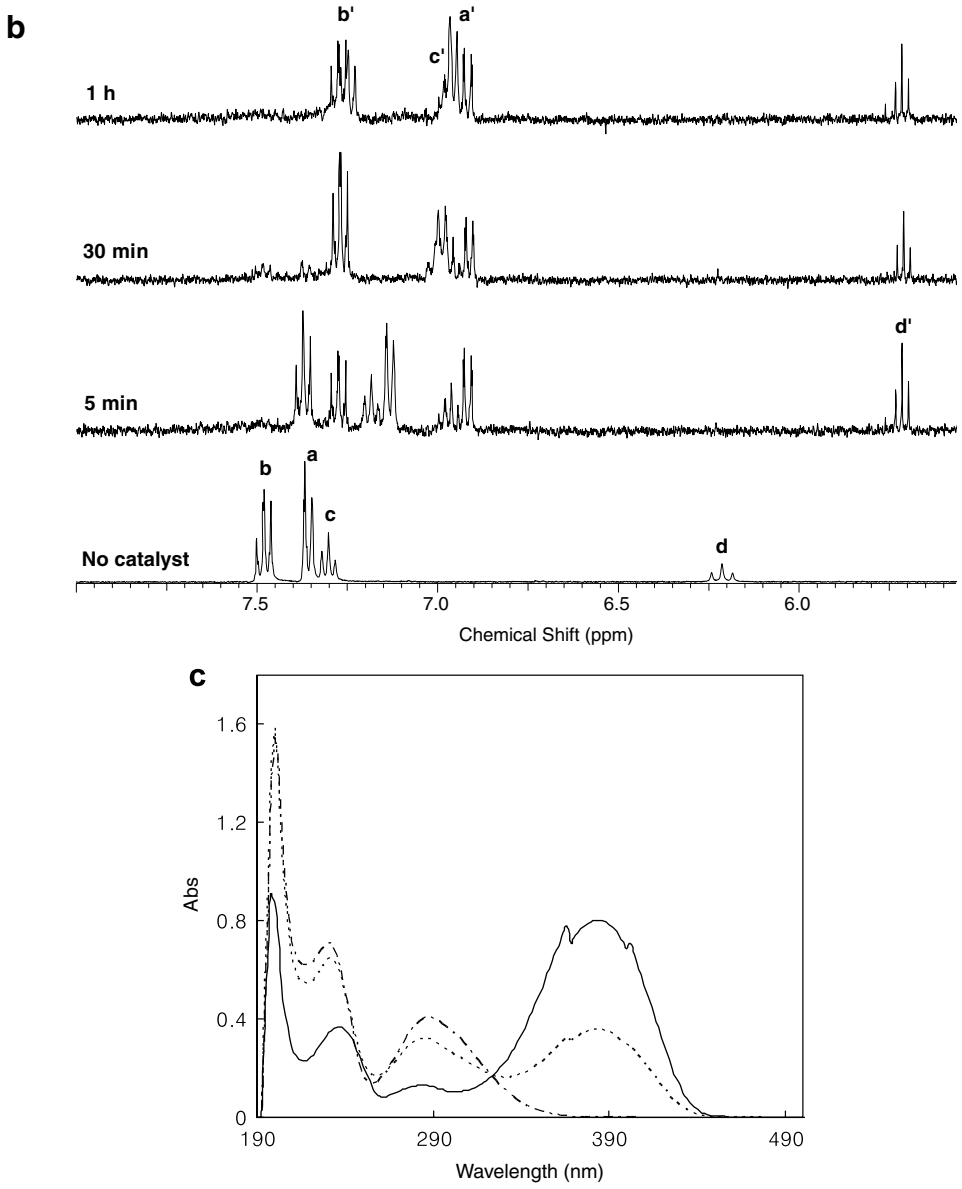

Figure 3. (*continued*)

Table 1. The synthesis of α-aminonitriles catalyzed by α-C16 and the succinoglycan monomers (M1–M3) in the mixture solutions of water and methanol

Catalysts	Substrates	Reaction time[a] (h)	Yield[b] (%)	ee[c] (%)
α-C16	**1**	1	100	*rac*
M2	**1**	1	100	*rac*
M1	**2**	3	48	54
	3	1	17	58
M3	**2**	3	40	55
	3	1	15	57

[a] Time that the reaction is completed.
[b] Determined by ^{1}H NMR analysis.
[c] The values were determined by HPLC analysis using a chiral column.
[d] See Section 1 for the detail conditions.

MeOH of HPLC grade were used as the reaction solutions in this study. A mixture of imine compounds (13.5 mM), TMSCN (14 mM) and the carbohydrates (1.3 mM) was stirred in the solution of water and MeOH at 30 °C. The reaction solutions for the substrates were composed of 1:1 ratio of water and MeOH, whereas of 2:8 ratio for **3** due to its very low solubility in pure water. The reactions were first monitored by TLC at 254 nm. Silica gel 60 F_{254} glass-backed TLC plates were spotted with the reaction mixtures and developed with the solvent system, Hex:EtOAc:MeOH = 2:1:1, for the mixtures with substrate **1** and with the system, Hex:EtOAc = 2:1, for the mixtures with the other substrates.

^{1}H NMR spectroscopic analysis was performed on a Bruker AVANCE 400 or 500 spectrometry. To calculate

the quantitative yields with ^{1}H NMR analysis, aliquots in each reaction mixture were taken at a period of time and MeOH was partially removed under a nitrogen gas, and then the mixtures were lyophilized. The NMR measurement for mixture of the substrate **1** with α-C16 or M2 was done in CD_3OD (99.8 atom % D) whereas those with the other substrates were in CD_3Cl (99.8 atom % D).

The ee values were also checked by HPLC analysis with a chiral column (Pirkle (*R*,*R*) Whelk-O1, Regis, USA) with Hex:IPA = 95:5 or 90:10 as solvent system.

1.2. Preparation and identification of α-C16 and the succinoglycan monomers

X. oryzae pv. *oryzae* KACC 10331 were from the Korean Agricultural Culture Collection (KACC), and they were grown in a TGY medium[12] at 28 °C with 150 rpm agitation. The microorganisms were grown for 2 days and collected by centrifugation at 4 °C for 10 min at 8000 rpm. The cell pellets were extracted with 5% trichloroacetic acid, and after centrifugation, the supernatant was neutralized with NH_4OH[13] and chromatographed on a Sephadex G-25 (2.5 × 34 cm) column at a rate of 1 mL/min. The fractions containing the putative cyclic glucan were pooled, concentrated and then applied to a column (2 × 35 cm) of DEAE-Sephadex to separate the anionic form of α-C16. Neutral one is collected and desalted on a Bio-Gel P-4 column (Bio-Rad). The column (2.4 × 54 cm) was eluted at room temperature with distilled water at a flow rate of 18 mL/h and then the desalted material was finally lyophilized. Structural analyses of the purified α-C16 were performed with FT-NMR spectroscopy and MALDI-TOF mass spectrometer using 2,5-dihydroxybenzoic acid (DHB) as a matrix in the positive mode.

To prepare the succinoglycan monomers, *R. meliloti* 1021 was cultured in 500 mL of GMS medium at 30 °C for 5 days.[10] Cells were harvested (13,000*g* for 10 min) and washed with 25 mL of GMS salts buffer at pH 7.0 and culture supernatants were frozen. After thawing, culture supernatants were concentrated 5-fold by rotary evaporation. Next, high-molecular-weight succinoglycan was precipitated from concentrated supernatants by adding 3 volumes of ice-cold ethanol. High-molecular-weight succinoglycans were then removed from concentrated supernatants by centrifugation (12,000*g* for 10 min). The supernatant was once more concentrated 5-fold by rotary evaporation and low-molecular-weight succinoglycans were obtained by adding 7 volumes of ice-cold ethanol and centrifugation.

The precipitate was dissolved in distilled water and samples were applied to a Bio-Gel P6 column (1 × 120 cm) which was eluted at room temperature with 0.5% AcOH at a rate of 20 mL/h. Fractions (1 mL) were collected and assayed for carbohydrate content. Material eluting in the position expected for the succinoglycan monomers from Low-molecular weight succinoglycan was pooled and then applied to a column (2 × 45 cm) of Bio-Gel P4 to separate the succinoglycan monomers at a rate of 30 mL/h. The fraction of monomer was pooled, concentrated, and subsequently desalted using a Sephadex G-15 column (1 × 49 cm). The M1, M2 and M3 fractions of the succinoglycan monomers were separated by Bio-Gel P6, and Bio-Gel P4 chromatography were further fractionated on a column (1.5 × 48 cm) of DEAE Sephadex that was pre-equilibrated with 5 mM KCl in a MOPS [3-(*N*-morpholino) propanesulfonic acid] buffer (10 mM; pH 7.0). The sample was loaded onto the column and eluted with KCl linear gradients by 400 mL of 5–250 mM KCl. Fractions (4 mL) were collected and assayed by the phenol–sulfuric acid method. Peaks were pooled, thoroughly dialyzed against water with a SpectraPor dialysis membrane tube having molecular-weight cutoff of 1000 and lyophilized. Then the purified succinoglycan monomers were confirmed through a Brucker 500 MHz NMR spectroscopy.

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