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Enzymatic Synthesis of ω-Hydroxyalkyl and n-Alkyl β-D-Galactopyranosides by the Transglycosylation Reaction of β-Galactosidase

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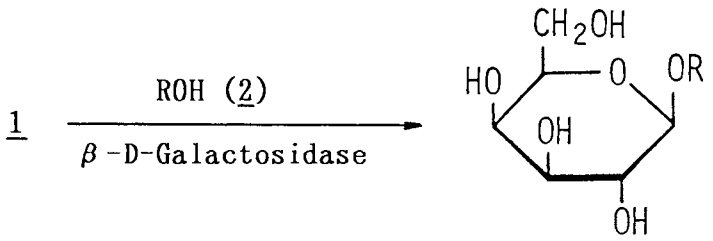
ω-Hydroxyalkyl and n-alkyl β-D-galactopyranosides were directly prepared in high yield from 1,8-octanediol, 1,10-decanediol, 1-octanol and 1-decanol by the transglycosylation reaction of *o*-nitrophenyl β-D-galactopyranoside (ONPG) or lactose using β-galactosidase.

Higher alkyl glycosides are of particular interest because in addition to their potential industrial, biological and pharmaceutical applications,[1] they are made from naturally abundant and renewable resources of fatty alcohols and sugars. From an ecological and an energy point of view alkyl glycoside will be a most promising novel nonionic surfactant in the next generation. Their preparation by chemical methods is widely used, but it was cumbersome and generally gave low yield. Furthermore, simultaneous formation of α- and β-anomers was observed as a disadvantage of the chemical method. In the biological field, homogeneity of surfactants chemical structure was strongly requested. The enzymatic method will be the most feasible for the direct and specific preparation of alkyl glycosides. Recently, some alkyl β-D-glucosides,[2] β-D-galactosides[3] and β-D-xylosides,[4] having relatively short alkyl chains, were prepared by the enzymatic transglycosylation reaction. However, there was no satisfactory method for the synthesis of higher alkyl glycosides.

In this communication, ω-hydroxyalkyl and n-alkyl β-D-galactopyranosides were directly prepared by the transglycosylation reaction of *o*-nitrophenyl β-D-galactpyranoside (ONPG) or lactose with 1,8-octanediol, 1,10-decanediol, 1-octanol and 1-decanol using β-galactosidase in a phosphate buffer containing acetone as a solvent. Interfacial and biological properties of the chemically synthesized n-alkyl β-D-galactopyranosides have been reported elswhere.[5]

β-Galactosidase (EC 3.2.1.23) from *Escherichia coli* was purchased from Sigma Chem. Co., and their activities for ONPG and lactose were 388 and 69 units/mg protein, respectively. The synthesis of 8-hydroxyoctyl



1 : ONPG / lactose

3	a	b	c	d
R	$HO(CH_2)_8-$	$HO(CH_2)_{10}-$	$n-C_8H_{17}-$	$n-C_{10}H_{21}$

β-D-galactopyranoside (3a) is described as a typical example. A mixture of ONPG (120.5 mg, 0.40 mmol) and 1,8-octanediol (584.9 mg, 4.0 mmol) was dissolved in a mixed solvent consisting of 0.067 $mol \cdot dm^{-3}$ phosphate buffer (pH 6.4, 8 mL) and acetone (2 mL), then β-galactosidase from *E. coli* (240 units, 0.62 mg) was added to the solution and stirred at 30℃ for 30 min. After the reaction, the solvent was evaporated in vacuo, the precipitated excess 1,8-octanediol was filtered off, the filtrate was further evaporated in vacuo and the precipitated diol was again filtered off to give a syrupy product comprising 3a, liberated galactose and the unreacted diol. This was added to acetone and precipitated galactose was filtered off and the filtrate was further purified by repeated recrystallization from acetone to give 3a as a white crystal(mp 123 - 124℃. $[\alpha]_D^{25}$ -15.3° (c 1, MeOH)) . The isolated product was analyzed by HPLC, elemental analysis, IR, ^1H NMR and ^{13}C NMR spectroscopy.[6] These spectral data for 3c and 3d agreed completely with the authentic compounds.[5]

10-Hydroxydecyl, octyl, and decyl β-D-galactopyranosides were also prepared using β-galactosidase from *E. coli*. Their reaction conditions and yields are shown in Table 1. It was confirmed from the HPLC analysis that the enzymatic glycosylation of ONPG with α,ω-diols gave exclusively mono β-galactopyranoside of the diols, and neither α,ω-digalactopyranoside nor the α-anomer was detected in the reaction mixture. This selectivity will be one of the prominent advantages over the conventional chemical method. The HPLC analysis with the calibration of authentic standards allowed us to periodically estimate the composition of the enzymatic reaction mixture.[7] Figure 1 shows the yield of 3a, as well as unreacted ONPG and liberated D-galactose versus reaction time. From this, it was indicated that the synthesis of alkyl galactopyranoside from ONPG and an alkanol by the

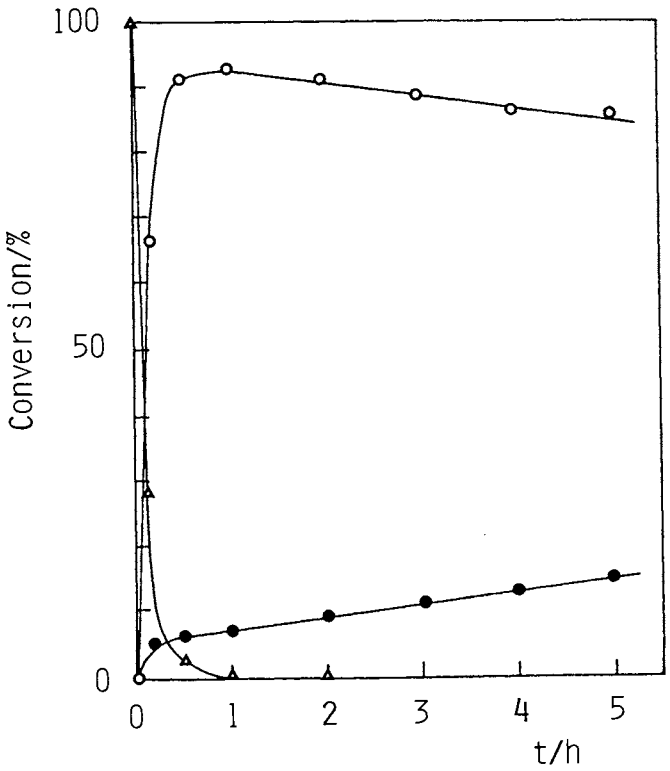


Fig. 1. Enzymatic preparation of 8-hydroxyoctyl β-D-galactopyranoside (3a) from ONPG and 1,8-octanediol with β-galactosidase from *E. coli*. Reaction conditions for 3a, same as those of Table 1.
○ : 3a, △ : unreacted ONPG, ● : liberated D-galactose

Table 1. Typical Preparation of Alkyl β-D-Galactopyranoside (3)[a)]

Compound	1	2	Mixed solvent (vol% of acetone in phosphate buffer)	t/h	Yield/%
3a	ONPG	$HO(CH_2)_8OH$	20	1.0	92.3
3a	Lactose	$HO(CH_2)_8OH$	20	8.0	49.6
3b	ONPG	$HO(CH_2)_{10}OH$	60	2.0	77.8
3c	ONPG	$C_8H_{17}OH$	55	0.5	50.4
3d	ONPG	$C_{10}H_{21}OH$	60	1.5	26.7

a) A mixture of 1 (0.40 mmol) and 2 (4.0 mmol) in a mixed solvent (10 mL) was stirred in the presence of β-D-galactosidase (240 units for ONPG / 85 units for lactose) at 30 ℃.

transglycosylation of β-galactopyranoside is competing with the hydrolysis reaction of ONPG. Within the first hour of the reaction, the rapid formation of 3a was observed along with a sharp decrease in unreacted ONPG. The maximum yield of 3a was attained after 30 min, then the product was slowly hydrolyzed by the enzyme with gradual formation of galactose. Similar tendencies were observed for the reaction of ONPG and 10-hydroxydecanol.

Lactose was also transglycosylated with α,ω-alkanols by β-galactosidase under similar reaction conditions as described for the α,ω-alkanols with ONPG to yield ω-hydroxyalkyl β-D-galactopyranoside in considerable yield. Maximum yield for 3a was 49.6%, but both the yield and the rate of reaction were slightly poorer than those of ONPG. Table 1 shows the typical results of the transglycosylation reaction of lactose and 1,8-octanediol.

This enzymatic method for the preparation of alkyl galactopyranoside will be useful both for industrial and pharmaceutical applications.

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6) 3a : ^{13}C-NMR(CD_3OD) ; δ 105.8(C1 of the pyranose ring), 77.3(C5), 75.8(C3), 73.3(C2), 71.6(C1' of 8-hydroxyoctyl group), 71.2(C4), 63.8(C8'), 63.3(C6), 34.4(C7'), 31.3-31.6(C3'-C5'), 27.9(C2'), 27.6(C6') ; Anal. Found(%) : C, 54.68 ; H, 8.89. Calcd (%) for $C_{14}H_{28}O_7$: C, 54.53 ; H, 9.15 ; $[\alpha]_D^{25}$ -15.3° (c 1, MeOH). 3b : ^{13}C-NMR(CD_3OD) ; δ 105.7(C1 of the pyranose ring), 77.3(C5), 75.8(C3), 73.3(C2), 71.6(C1' of 10-hydroxydecyl group), 71.1(C4), 63.8(C10'), 63.2(C6), 34.4(C9'), 31.3-31.6(C3'-C7'), 27.9(C2'), 27.7(C8') ; Anal. Found(%) : C, 57.10 ; H, 9.17. Calcd (%) for $C_{16}H_{32}O_7$: C, 57.12 ; H, 9.59 ; $[\alpha]_D^{25}$ -17.1° (c 1, MeOH).
7) HPLC column : TOSOH Co. Ltd., TSKgel Amide-80 ; Eluent : acetonitrile/water = 75/25 ; Detection : refractive index (RI) detector (JASCO 830-RI).

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