[Chem. Pharm. Bull. 32(3)1183—1187(1984)]

Synthesis of β-Alkyl Glucosides by Enzymic Transglucosylation

NAOKI MITSUO, HIROSHI TAKEICHI, and TOSHIO SATOH*

School of Pharmacy, Tokushima University of Arts & Science, Yamashiro-cho, Tokushima 770, Japan

(Received June 29, 1983)

Lactase from *Kluyveromyces lactis* was found to have high transglucosylation activity, transferring the glucosyl group of aryl glucoside to an alcoholic hydroxyl group in high yield. Both primary and secondary alcohols can accept the glucosyl group. Transglucosylation to glycol yielded a single kind of glucoside. A very hydrophilic alcohol, such as glycerol, could not serve as a substrate in this system. Hydrophobic alcohols slightly soluble in water were glucosylated in a two-phase system consisting of water and an organic solvent immiscible with water.

Keywords——transglucosylation; transglycosylation; β-glucoside; lactase; *Kluyveromyces lactis*; phenyl β-D-glucopyranoside; hydrophobic alcohol; two-phase system; alkyl glucoside; enzymic transglucosylation

Glycosylation methods can be classified as chemical and enzymic.[1] The chemical methods, in general, have some disadvantages. One of these is that α- and β-anomers are formed simultaneously, and another is that poisonous heavy metal salts or expensive silver compounds have to be used. Furthermore, chemical methods involve many steps and cannot be applied to the glycosylation of aglycones which are labile to acids or bases. On the other hand, though enzymic methods do not suffer from these disadvantages, few examples of their application have been reported. In 1936, Veibel prepared glucosides by an enzymic method,[2a] using emulsin, glucose, and lower alcohols. His method, called direct glucosylation, is an equilibrium reaction and requires a high concentration of alcohol to obtain a glucoside. Consequently, its application is limited to lower alkyl glucosides. Later several papers[2b-h] on enzymic glycosylation appeared, but the methods are of limited value because the enzymes required are very expensive or difficult to obtain.

Recently Tanaka *et al.* studied enzymic glycosylation with some crude enzymes utilized in food manufacturing.[2i] In their study, α-glucoside was obtained in 21% yield, but the yield of β-glucoside was not high enough to be useful in industrial mass production. In their method, called transglycosylation, the reaction does not come to equilibrium, a high concentration of alcohol is not required, if an appropriate starting glycoside is used.

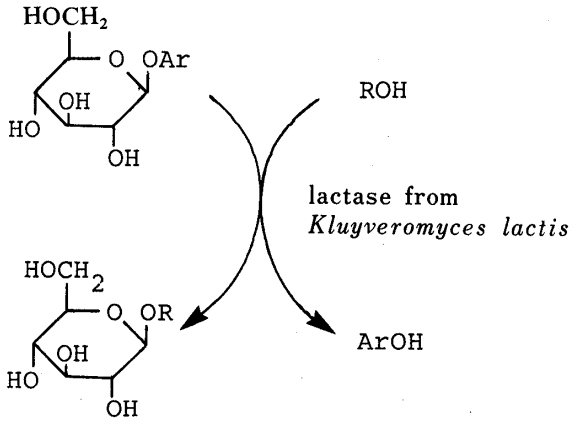
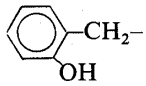
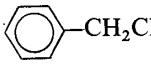


Chart 1

TABLE I. Yields of Alkyl β-D-Glucosides Obtained, Reaction Conditions, and Various Spectral Data for the Glucoside Acetates

Glucoside	Alkyl group (R)	Yield (%)	Alcohol concentration in transglucosylation (mmol of alcohol/ml of buffer)	mp of the acetate (°C)	NMR signal of anomeric proton of the acetate (δ, Hz)	Anal. of the acetate Calcd (Found) C	H
1a	CH_3-	67	8.0	a)	a)	a)	
2a	$CH_3(CH_2)_3-$	40	0.5	65.5—66.5	4.50 (d, J=7.5)	53.46 (53.59	6.98 7.16)
3a	$(CH_3)_2CH-$	24	1.0	137—138	4.40 (d, J=7.5)	52.30 (51.96	6.71 6.74)
4a		13	0.3	123	4.45 (d, J=7)	55.81 (55.73	7.03 7.30)
5a	$HOCH_2CH_2-$	36	1.0	50.5—51	4.55 (d, J=7)	49.77 (49.56	6.03 6.27)
6a	$(CH_3)_2C(OH)(CH_2)_3-$	13	0.76	b)	4.50 (d, J=8)	b)	
7a	$(CH_3)_2C(OH)CH_2CH(CH_3)-$	9	1.5	110—122	4.60 (0.5H, d, J=8)c) 4.65 (0.5H, d, J=8)c)	53.56 (53.41	7.19 7.19)
8a		27	0.26	d)	d)	d)	
9a		22	e)	71—72	4.55 (d, J=7)	58.41 (58.51	6.24 6.24)
10a	$CH_3(CH_2)_7-$	13 (17)f)	e)	61—62	4.50 (d, J=7.5)	57.38 (57.10	7.88 7.92)
11a	$CH_3(CH_2)_3\diagdown\diagup CH_3(CH_2)_3CH-$	5 (6)f)	e)	79—80	4.55 (d, J=8)	58.21 (58.40	8.07 8.22)

a) This glucoside was shown to be identical with authentic methyl β-D-glucopyranoside by direct comparison of mp (111—112.5 °C) and IR spectra, and by mixed mp determination.
b) The acetate was oily.
c) The acetate was a 1 : 1 mixture of the isomers at the 2-position of the aglycone.
d) The glucoside was identical with isosalicin (mp, IR spectrum, hydrolysis products, and analytical data) (see experimental section).
e) Two-phase transglucosylation.
f) Conversion ratio.

We studied enzymic transglycosylation to obtain various alkyl β-glucosides in high yields, and found that lactase from *Kluyveromyces lactis* for industrial use (so-called essential β-galactosidase[3]) has strong transglucosylation activity, and converts salicin or phenyl β-D-glucoside to alkyl β-D-glucosides (**1a**—**11a**).

Table I shows the isolated yields of the glucosides obtained, the reaction conditions, and various spectral data of the acetates.

Among simple alkyl glucosides, methyl, *n*-butyl, isopropyl, and cyclohexyl β-D-glucosides[4] (**1a**, **2a**, **3a**, **4a**) were synthesized by this system. Not only a primary but also a secondary alcohol can be utilized for transglucosylation, though, in general, the transglucosylation yield of a primary alcohol is higher than that of a secondary alcohol. Tertiary alcohols, *e.g. tert*-butanol, are not transglucosylated. The higher the alcohol concentration, the higher is the yield of the glucoside, but if the alcohol concentration is very high, both transglucosylation and hydrolysis are inhibited. The limit of concentration varies from case to case.

The relationship between transglucosylation reactivity and the hydrophilicity of an alcohol was studied by using polyhydric alcohols. Transglucosylation to ethylene glycol produced 2-hydroxyethyl β-D-glucoside (**5a**) to some extent. When glycerol or glucose was used as substrate, hydrolysis of the starting glucoside proceeded, but transglucosylation did not. It is considered that a very hydrophilic alcohol cannot be transglucosylated in this system.

Beta configuration of transglucosylation products was confirmed by the finding that these glucosides were easily hydrolyzed by β-glucosidase from almond. Furthermore, nuclear magnetic resonance (NMR) spectra of tetraacetates derived from these glucosides showed the coupling constants of the anomeric protons to be in the range of 7—8 Hz, supporting a diaxial disposition of the anomeric proton and C-2 proton.

The difference in reactivity to various types of hydroxyl groups was studied. It became apparent that the transglucosylation system acted on a primary or secondary hydroxyl group in preference to a tertiary or phenolic hydroxyl group. It is not necessary to protect tertiary or phenolic hydroxyl groups in transglucosylation to a dihydroxylic compound which has primary or secondary hydroxyl groups. In fact, a glucoside of the primary alcohol (**6a**) was obtained by transglucosylation to 4-methylpentane-1,4-diol. Transglucosylation to DL-4-methylpentane-2,4-diol (hexylene glycol) produced a glucoside of the secondary alcohol (**7a**). This product was a 1 : 1 mixture of D- and L-isomers at the 2-position of the aglycone. In the transglucosylation to salicyl alcohol, an alcoholic hydroxyl group was selected to yield isosalicin (**8a**).[5]

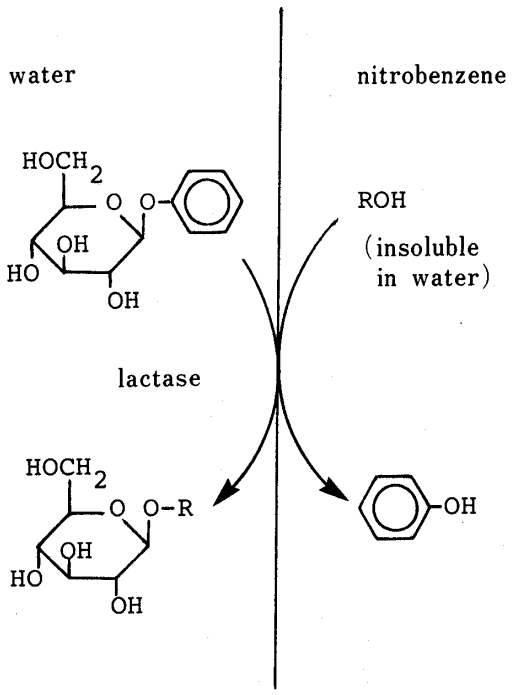


Chart 2

In the enzymic glycosylation method, the alcohols were limited to those soluble in water, such as lower alcohols or alcohols having some hydrophilic functional groups, because all the reactions were carried out in an aqueous solution. Enzymic transglycosylation of very hydrophobic alcohols sparingly soluble in water has been difficult. However, this difficulty was overcome by the use of a two-phase system (water and an organic solvent immiscible with water).

Thus, a hydrophobic alcohol such as phenethyl, octyl, or 5-nonyl alcohol was dissolved

in nitrobenzene, while phenyl β-glucoside and lactase were dissolved in a buffer solution. The two solutions were mixed and vigorously stirred. Enzymic transglucosylation occurred at the interface to yield phenethyl β-D-glucoside (**9a**), octyl β-D-glucoside (**10a**), or 5-nonyl β-D-glucoside (**11a**). This two-phase transglucosylation and concurrent hydrolysis of the starting or resultant glucoside proceeded more rapidly than the ordinary homogeneous enzymic reactions. The optimal temperature of the two-phase reaction is 50 °C, which is higher than that of usual homogeneous enzymic reactions. The preparation of useful steroid or terpenoid glucosides by two-phase transglucosylation is now under investigation.

Experimental

Melting points were determined on a Yanaco hot stage apparatus and are uncorrected. NMR spectra were obtained on a Hitachi R-42FT Fourier transform spectrometer (90 MHz) or a Varian EM360 NMR spectrometer (60 MHz). Chemical shifts are in ppm downfield from tetramethylsilane (TMS). Infrared (IR) spectra were recorded on a Hitachi 295 infrared spectrophotometer. Mass spectra (MS) were obtained with a Shimadzu-LKB-9000B gas chromatograph-mass spectrometer. Lactase from *Kluyveromyces lactis* was provided by Godo-Shusei Co., Ltd.; it is also commercially available. Phenethyl β-D-glucopyranoside was prepared from glucose in three steps according to the literature.[6a,b]

Typical Homogeneous Transglucosylation: Preparation of 2-Hydroxyethyl β-D-Glucopyranoside (5a)——Phenyl β-D-glucopyranoside (0.0512 g, 0.2 mmol), ethylene glycol (0.124 g, 2 mmol), and lactase from *Kluyveromyces lactis* (0.10 g) were dissolved in 0.1 M phosphate buffer (pH 7, 2 ml), and incubated at 35 °C for 0.5 h. Methanol (3 ml) was added to the solution to stop the enzymic reaction. The whole was left to stand overnight in a refrigerator, and the precipitate formed was removed by centrifugation. The supernatant was chromatographed on cellulose (*n*-BuOH sat. with H_2O) to give a glucoside **5a** (0.0160 g). The glucoside (**5a**, 0.023 g) was completely hydrolyzed to glucose and ethylene glycol with β-glucosidase from almond (0.006 g) in 0.1 M phosphate buffer (pH 4.5, 1.5 ml) at 35 °C within 3.5 h. 2-Acetoxyethyl β-D-glucopyranoside tetraacetate (**5b**) was obtained by treatment of **5a** with pyridine/Ac_2O at room temperature for half a day. It was recrystallized from diisopropyl ether. IR (KBr): 2950, 1745, 1220, 1035 cm^{-1}. NMR ($CDCl_3$) δ 2.00—2.10 (15H, Ac), 3.75 (2H, t, J=5.5 Hz, CH_2CH_2OAc), 4.20 (2H, t, J=5 Hz, CH_2CH_2OAc). MS *m/e*: 375 $(M-OAc)^+$, 361 $(M-CH_2OAc)^+$, 331 $(M-OCH_2CH_2OAc)^+$.

4-Hydroxy-4-methylpentyl β-D-Glucopyranoside 2,3,4,6-Tetraacetate (6b)——The glucoside **6a** was acetylated with pyridine/acetic anhydride as usual to give oily **6b**. The tertiary hydroxyl group was not acetylated. IR (neat): 3400, 2960, 1750, 1220, 1030 cm^{-1}. NMR ($CDCl_3$) δ 1.25 (6H, s, $(CH_3)_2C$), 1.60 (2H, m, $CH_2C(CH_3)_2$, 1.75 (2H, m, $CH_2CH_2C(CH_3)_2$), 2.00—2.10 (12H, Ac), 3.75 (2H, m, $OCH_2CH_2CH_2C(CH_3)_2$).

3-Hydroxy-1,3-dimethylbutyl β-D-Glucopyranoside 2,3,4,6-Tetraacetate (7b)——The pentahydric compound **7a** was acetylated with pyridine/acetic anhydride as usual to give **7b**. Recrystallization from methylene chloride/petroleum ether gave an analytical sample. IR (KBr): 3580, 2985, 1750, 1230, 1040 cm^{-1}. NMR ($CDCl_3$) δ 1.20 (9H, m, CH_3), 1.60 (3H, m, $CHCH_2C(CH_3)_2$), 2.00—2.10 (12H, Ac), 4.10 (1H, m, $CHCH_2C(CH_3)_2$). MS *m/e* 331 $(M-OC_6H_{12}OH)^+$.

Isosalicin (8a)——A sample of **8a** was hydrolyzed with β-glucosidase to give glucose and salicyl alcohol. Crude **8a** was recrystallized from water, and dried under a vacuum at 50 °C for 3 h, mp 67—69 °C (lit.[5] 68 °C), IR (KBr): 3400, 3200, 2800, 1615, 1595, 1505, 1460, 1015, 755 cm^{-1}. *Anal.* Calcd for $C_{13}H_{18}O_7 \cdot 2/3H_2O$: C, 52.35; H, 6.53. Found: C, 52.16; H, 6.62.

Typical Two-Phase Transglucosylation Phenethyl β-D-Glucopyranoside (9a)——Phenethyl alcohol (2.44 g, 20 mmol) in nitrobenzene (20 ml) was added to a 0.1 M phosphate buffer solution (pH 7, 20 ml) of phenyl β-D-glucopyranoside (0.512 g, 2 mmol) and the lactase (0.40 g). After vigorous stirring for 1 h at 50 °C, the reaction mixture was poured into a cold mixture of water (70 ml) and carbon tetrachloride (70 ml), and the whole was shaken. Methanol (180 ml) was added to the water layer, and the whole was allowed to stand for 1 h at room temperature, then centrifuged. The supernatant was evaporated *in vacuo*, and the residue was dissolved in a mixed solvent ($CHCl_3$/C_2H_5OH/H_2O : 5/2/2, upper layer) followed by extraction with the lower layer to remove the glucose. Evaporation of the combined lower layer *in vacuo* followed by silica gel chromatography of the residue ($CHCl_3$/CH_3OH/H_2O : 16/4/1, lower layer) gave oily **9a** (0.124 g). A sample of **9a** was hydrolyzed to glucose and phenethyl alcohol by β-glucosidase. Acetylation of **9a** in the usual way gave its tetraacetate (**9b**) as colorless crystals, which were recrystallized from 45% aqueous ethanol. IR (KBr): 1740, 1600, 1580, 1500, 1460, 1220 cm^{-1}. MS *m/e*: 347 $(M-CH_2CH_2Ph)^+$, 331 $(M-OCH_2CH_2Ph)^+$.

References and Notes

1) N. Mitsuo, *Farumashia*, **18**, 628 (1982).

2) *a*) S. Veibel, *Enzymologia*, **1**, 124 (1936); *b*) L. G. Witby, *Biochem. J.*, **50**, 433 (1952); *c*) W. J. Whelan and D. M. Jones, *ibid*, **54**, 34 (1953); *d*) W. R. Fetzer, E. K. Crosby, C. E. Engel, and L. C. Kirst, *Ind. Eng. Chem.*, **45**, 1057 (1953); *e*) J. H. Hash and K. W. King, *J. Biol. Chem.*, **232**, 395 (1958); *f*) J. B. Pridham, *Chem. Ind.* (London), **1961**, 1172; *g*) W. Boos, J. Lehmann, and K. Wallenfels, *Carbohydr. Res.*, **1**, 419 (1966); *h*) S. C. Pan *Biochemistry*, **9**, 1833 (1970); *i*) K. Itano, K. Yamasaki, C. Kihara, and O. Tanaka, *Carbohydr. Res.*, **87**, 27 (1980).
3) R. C. Dickson, L. R. Dickson, and J. S. Markin, *J. Bacteriol.*, **137**, 51 (1979).
4) B. Lindberg, *Acta Chem. Scand.*, **3**, 151 (1949).
5) M. Tabata, F. Ikeda, N. Hiraoka, and M. Konoshima, *Phytochemistry*, **15**, 1225 (1976).
6) *a*) R. L. Whistler and M. L. Wolfrom (eds.), "Methods in Carbohydrate Chemistry," Vol. II, Academic Press, New York, 1963, p. 212, 216; *b*) B. Helferich and E. Hillebrecht, *Ber.*, **66**, 378 (1933).