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# Enzymic Synthesis of Glycosides of Racemic Alcohols Using β-Galactosidase and Separation of the Diastereomers by High-Performance Liquid Chromatography Using a Conventional Column[1)]

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β-Glycosides of phenylethylene glycol, propylene glycol, 3-chloropropylene glycol and 1-phenylethyl alcohol were synthesized by transglycosidation using β-galactosidase from *Aspergillus oryzae*, and the diastereomers were resolved by high-performance liquid chromatography on a conventional column.

**Keywords**——enzymic transglycosidation; β-galactosidase; *Aspergillus oryzae*; racemic alcohol resolution; HPLC

Optically active alcohols are important synthons for the synthesis of various natural products.[2)] Resolution of the racemate is a convenient method to obtain them, if the racemate is readily available. Two methods have previously been employed for this purpose. The first is the formation of the ester or urethane derivative of the alcohol by using an optically active acid or isocyanate; the diastereomeric mixture is then separated by fractional crystallization or chromatography.[3)] The second method involves direct separation of the racemate by column chromatography using an optically active stationary phase.[4a,b)] However, the former method is a tedious procedure and cannot be applied to all kinds of alcohols. Furthermore it is not always possible to obtain 100% ee in one operation. The latter method requires a very expensive column and is limited to compounds which possess an aromatic ring. In this paper, we report a more general two-stage method for the resolution of alcohols. The alcohol is converted enzymatically to a mixture of diastereomeric glycosides, which is separated by high-performance liquid chromatography (HPLC). Each glycoside is then subjected to enzymatic hydrolysis to afford the pure optically active alcohol. The alcohols used in this study were phenylethylene glycol, propylene glycol, 3-chloropropylene glycol, and α-phenylethyl alcohol. The β-glycosides of the racemic alcohols were synthesised by our method of transglycosylation using β-galactosidase from *Aspergillus oryzae*.[5,6a,b)] As α-phenylethyl alcohol is only slightly soluble in water, aqueous acetonitrile was used for the synthesis of glycosides of α-phenylethyl alcohol. During the synthesis of 2-*O*-β-D-galactosyl phenylethylene glycol (Ia), 2-*O*-β-D-glucosyl-phenylethylene glycol (Ib), and 1-*O*-β-D-glucosyl-propylene glycol (II), small amounts of the corresponding glycosides of the secondary hydroxyl groups were formed. However, when 1-*O*-β-D-glucosyl-3-chloropropylene glycol (III) was synthesized, none of the secondary alcohol glycoside could be detected (Table I). The diastereomeric pairs were almost completely separated using a Nucleosil $_{10}C_{18}$ or a Chemcosorb $_{7}C_{8}$ column. The resolution factor $R$ for the β-galactoside (Ia) was 2.17 while that of the β-glucoside (Ib) was 2.99. Thus, the β-glucosides are more easily separated than the corresponding β-galactosides (Figs. 1 and 2). The successful separation of the β-glucosides (II) and (III) indicates that it is possible to resolve aliphatic glycols, the resolution of which is normally difficult, *via* their glycosides.

TABLE I. Yields and Liquid Chromatographic Separation of Glycosides of Racemic Alcohols

| Glycosides of racemic alcohol | | Yield[a] (%) | $K'$[f] and (absolute configuration) less retained | more retained | $\alpha$[g] | $R$[h] |
|---|---|---|---|---|---|---|
| 2-*O*-β-D-Galactosyl-phenylethylene glycol | (Ia)[b] | 20.4 | 9.86 (1*R*) | 10.95 (1*S*) | 1.11 | 2.17 |
| 2-*O*-β-D-Glucosyl-phenylethylene glycol | (Ib)[b] | 38.8 | 14.23 (1*R*) | 16.13 (1*S*) | 1.13 | 2.99 |
| 1-*O*-β-D-Glucosyl-propylene glycol | (II)[c] | 47.9 | 2.99 (2*R*) | 3.26 (2*S*) | 1.09 | 1.33 |
| 1-*O*-β-D-Glucosyl-3-chloropropylene glycol | (III)[d] | 43.1 | 4.88 (2*S*) | 5.45 (2*R*) | 1.12 | 1.63 |
| 1-*O*-β-D-Glucosyl-α-phenylethyl alcohol | (IV)[e] | 5.9 | 14.51 (1*S*) | 15.50 (1*R*) | 1.07 | 1.15 |

*a*) Yields were based on the phenyl β-D-glycosides. HPLC conditions were as follows. *b*) Column, Chemcosorb $_7C_8$ 300 × 4.6 mm (i.d.); flow rate of $CH_3CN : H_2O$ (5 : 95), 1 ml/min. *c*) Column, Chemcosorb $_7C_8$ 300 × 4.6 mm (i.d.); flow rate of $H_2O$, 1 ml/min. *d*) Column, Chemcosorb $_7C_8$ 300 × 4.6 mm (i.d.); flow rate of $H_2O$, 1.5 ml/min. *e*) Column, Nucleosil $_{10}C_{18}$ 600 × 8 mm (i.d.); flow rate of $CH_3CN : H_2O$ (8 : 92), 4 ml/min. *f*) Capacity factor = (retention volume of an enantiomer − void volume of column)/(void volume of column). *g*) Separation factor = ($K'$ of more retained enantiomer)/($K'$ of less retained enantiomer). *h*) Resolution factor = 2 × (distance between the peaks of more and less retained enantiomers)/(sum of bandwidth of the two peaks).

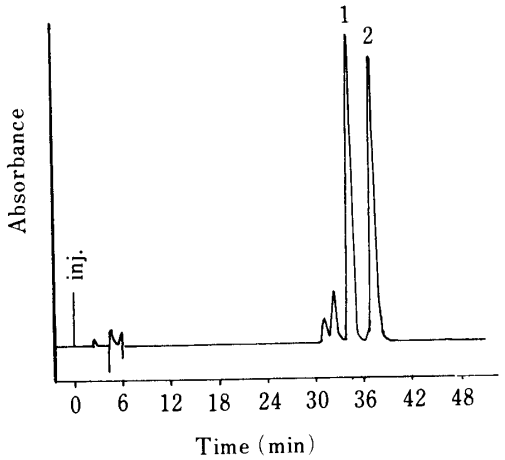



Fig. 1. Separation of the Diastereomers of 2-*O*-β-Galactosyl-phenylethylene Glycol (Ia)

1, (1*R*); 2 (1*S*); detector, UV at 254 nm.

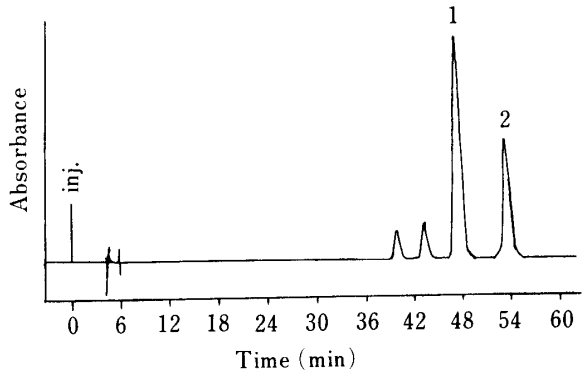



Fig. 2. Separation of the Diastereomers of 2-*O*-β-Glucosyl-phenylethylene Glycol (Ib)

1, (1*R*); 2 (1*S*); detector, UV at 254 nm.

Although α-phenylethyl alcohol possesses an aromatic ring, it has not been resolved by using an optically active resolution column. However, the glycosides (IV) could be separated on a conventional column (Fig. 3). Hydrolysis of each β-glycoside under mild conditions afforded an optically active alcohol of high purity, *e.g.* (*S*)-(Ia) and (*S*)-(Ib) were hydrolyzed using β-galactosidase (the same enzyme as was used for the synthesis of the glycosides) to give (*S*)-phenylethylene glycol of approximately 100% ee. Using this method, it may be possible to resolve previously unresolved alcohols, which may be useful in the synthesis and configurational analysis of various natural products.

The configuration of the alcohol moiety of II was determined by direct comparison with an authentic sample prepared from (*S*)-propylene glycol by this enzymic method. The (*R*) and (*S*) configurations of the alcohol moiety of IV were determined by HPLC in comparison with authentic samples synthesized from (*R*) and (*S*)-α-phenylethyl alcohols. The absolute configuration of the aglycone of III was determined as follows. The front peak substance of

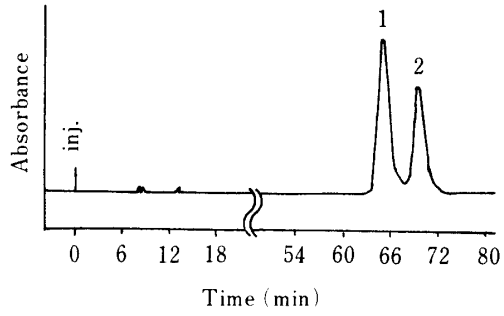
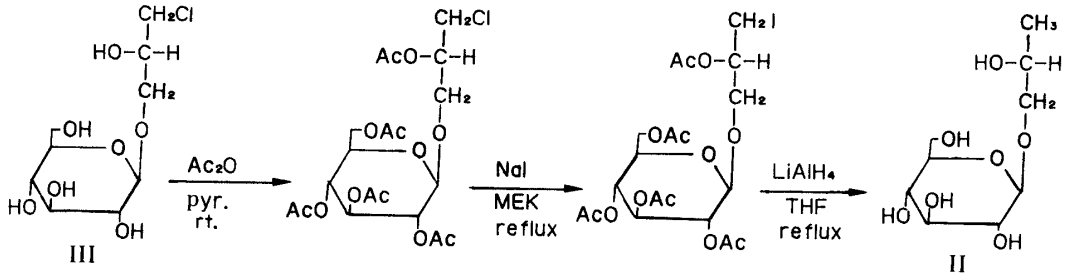



Fig. 3. Separation of the Diastereomers of 1-*O*-*β*-Glucosyl-α-phenylethyl Alcohol (IV)

1, (1*S*); 2 (1*R*); detector, UV at 254 nm.




MEK = methyl ethyl ketone

Chart 1

III was collected by HPLC and was converted to II *via* three steps as shown in Chart 1. As this sample was identical with (2*R*)-II, the alcohol moiety of III (front peak substance) was determined to have (2*S*) configuration.

## Experimental

Melting points were determined on a Yanaco hot stage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL GX-400 (400 MHz) instrument. Chemical shifts are expressed in ppm downfield from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Mass spectra (MS) were measured with a Shimadzu-LKB 9000 B gas chromatograph-mass spectrometer. Infrared (IR) spectra were recorded on a Shimadzu IR-27G spectrometer. Optical rotations were determined on a DIP-140 polarimeter. HPLC was performed (at room temperature) on a Waters 6000A instrument with a Nucleosil $_{10}C_{18}$ column (600 × 8 mm i.d.) or Chemcosorb $_{7}C_{8}$ column (300 × 4.6 mm i.d.) and an ultraviolet (UV) detector (UVIDEC 100-IV) or a refractive index (RI) detector (R-401). The UV detector was set at 254 nm. The crude *β*-galactosidase prepared from *Asp. oryzae* has a specific activity of 36 units/mg[7] (*β*-glucosidase activity 0.14 units/mg[8]). This crude preparation was kindly supplied by Kohjin Co., Ltd.[9] Phenyl *β*-D-galactoside and phenyl *β*-D-glucoside were prepared from the corresponding sugars in three steps according to the literature.[10a,b]

**Synthesis and Resolution of 2-*O*-*β*-D-Glucosyl-phenylethylene Glycol**——Phenyl *β*-D-glucoside (5.12 g, 20 mmol) and phenylethylene glycol (11.04 g, 80 mmol) were dissolved in phosphate buffer (0.1 M, pH 5, 160 ml), and incubated at 35 °C. Phosphate buffer (0.1 M, pH 5, 40 ml) containing 31800 units[7] of *β*-galactosidase was then added and the resulting mixture was allowed to stand at 35 °C for 180 min. Aqueous sodium carbonate (0.1 M, 100 ml) was added to stop the enzymatic reaction and the solution was then passed through a short cellulose column. Concentration of the eluate and chromatography of the residue on silica gel ($CHCl_3$–MeOH (5 : 1)) gave 2-*O*-*β*-D-glucosyl-phenylethylene glycol (Ib) (2.32 g). The products were separated by HPLC at room temperature on a Chemcosorb $_{7}C_{8}$ column (300 × 4.6 mm i.d.) with a UV detector set at 254 nm. The column was washed with $CH_3OH$–$H_2O$ (5 : 95) at a flow rate of 4.5 ml/min to give the glucosides.

**2-*O*-*β*-D-Glucosyl-(1*R*)-phenylethylene Glycol**——Amorphous powder. mp 59—61 °C. $[\alpha]_D^{26}$ −4.8° (*c* = 0.978, $H_2O$). IR(KBr): 3380, 2925, 1630, 1490, 1450, 1160, 1070 $cm^{-1}$. NMR ($D_2O$) *δ*: 3.31 (1H, dd, *J* = 8.6, 7.8 Hz, H-2′), 3.39 (1H, t, *J* = 9.0 Hz, H-4′), 3.44 (1H, ddd, *J* = 9.0, 5.6, 2.6 Hz, H-5′), 3.50 (1H, t, *J* = 9.2 Hz, H-3′), 3.72 (1H, dd, *J* = 12.2, 5.6 Hz, H-6′), 3.88 (1H, dd, *J* = 11.0, 3.9 Hz, –CH(OH)–C<u>H</u>$_2$–O–), 3.91 (1H, dd, *J* = 12.2, 2.6 Hz, H-6′), 4.07 (1H, dd, *J* = 11.0, 7.8 Hz, –CH(OH)–C<u>H</u>$_2$–O–), 4.51 (1H, d, *J* = 7.8 Hz, H-1′), 4.99 (1H, dd, *J* = 7.8, 3.9 Hz, Ph-C<u>H</u>(OH)–), 7.41—7.46 (5H, aromatic protons). *Anal.* Calcd for $C_{14}H_{20}O_7 \cdot H_2O$: C, 52.83; H, 6.97. Found: C, 53.07; H, 6.84.

**2-*O*-*β*-D-Glucosyl-(1*S*)-phenylethylene Glycol**——Amorphous powder. mp 68—70 °C. $[\alpha]_D^{26}$ +15.11° (*c*=0.966, $H_2O$). IR(KBr): 3400, 2910, 1630, 1495, 1430, 1165, 1075 $cm^{-1}$. NMR ($D_2O$) $\delta$: 3.33 (1H, d, *J*=9.0 Hz, H-2′), 3.39 (1H, t, *J*=9.3 Hz, H-4′), 3.46 (1H, dd, *J*=9.1, 5.4 Hz, H-5′), 3.50 (1H, d, *J*=9.0 Hz, H-3′), 3.72 (1H, dd, *J*=12.2, 5.4 Hz, H-6′), 3.83 (1H, dd, *J*=10.6, 8.3 Hz, –CH(OH)–C<u>H</u>$_2$–O–), 3.91 (1H, d, *J*=12.2 Hz, H-6′), 4.08 (1H, dd, *J*=10.6, 3.5 Hz, –CH(OH)–C<u>H</u>$_2$–O–), 4.51 (1H, d, *J*=8.1 Hz, H-1′), 4.99 (1H, dd, *J*=8.3, 3.5 Hz, Ph-C<u>H</u>(OH)–), 7.42—7.45 (5H, aromatic protons). *Anal.* Calcd for $C_{14}H_{20}O_7 \cdot H_2O$: C, 52.83; H, 6.97. Found: C, 52.79; H, 6.81.

**Hydrolysis of 2-*O*-*β*-D-Glucosyl-(1*S*)-phenylethylene Glycol**——2-*O*-*β*-D-Glucosyl-(1*S*)-phenylethylene glycol (45 mg, 0.15 mmol) was dissolved in phosphate buffer (0.1 M, pH 5, 0.5 ml) and incubated at 35 °C. Phosphate buffer (0.1 M, pH 5, 1 ml) containing 320 units of *β*-galactosidase from *Asp. oryzae* was then added and the resulting mixture was left at 35 °C for 40 h. Aqueous sodium carbonate (0.1 M, 0.8 ml) was added and the mixture was centrifuged. The supernatant was extracted with benzene and the solvent was evaporated off to give phenylethylene glycol (6.4 mg, y. 33%), which was recrystallized from benzene. mp 61—63 °C. IR(KBr): 3400, 2850, 1600, 1490, 1450, 1190, 1090, 1065, 1020 $cm^{-1}$. MS *m/z*: 138 ($M^+$), 108, 79, 77. $[\alpha]_D^{19}$ +40.5° (*c*=1.050, EtOH), (lit.[11] $[\alpha]_D^{20}$ +40.6° (*c*=3.23, EtOH)).

**Determination of the Absolute Configuration of 1-*O*-*β*-D-Glucosyl-3-chloropropylene Glycol**——1-*O*-*β*-D-Glucosyl-3-chloropropylene glycol pentaacetate (174 mg, 0.36 mmol, y. 98%) was obtained by treatment of (III) (100 mg, 0.37 mmol) (front peak) with pyridine–$Ac_2O$ at room temperature for 1 d. Methyl ethyl ketone (MEK) solution containing anhydrous sodium iodide (17 mg, 0.11 mmol) was refluxed for 1 h, after which glucosyl 3-chloropropylene glycol pentaacetate (40 mg, 0.083 mmol) was added to it.[12] The mixture was heated under reflux for 37 h, then cooled to room temperature and filtered. The residue was washed with MEK, and the washings and filtrate were combined and evaporated. The residue was dissolved in chloroform and this solution was washed with 10% sodium hydrogen sulfite solution, 5% sodium hydrogen carbonate solution and water. The organic layer was dried over anhydrous magnesium sulfate for 1 d, and the solvent was evaporated off to give 1-*O*-*β*-D-glucosyl-3-iodopropylene glycol pentaacetate (15.6 mg). Lithium aluminium hydride (6 mg, 0.158 mmol) was dissolved in tetrahydrofuran (THF) (2 ml), and 1-*O*-*β*-D-glucosyl-3-iodopropylene glycol (15.6 mg, 0.027 mmol) in THF (1 ml) was added to it. The mixture was refluxed for 40 h.[13a,b] After cooling of the solution, water was added to it cautiously to decompose excess hydride, and a few drops of 10% sulfuric acid were added. The solvent was evaporated off and the residue was analyzed by HPLC. As this sample corresponds to 1-*O*-*β*-D-glucosyl-(2*R*)-propylene glycol, the front peak of 1-*O*-*β*-D-glucosyl-3-chloropropylene glycol was determined to be the (2*S*) glycol.

## References and Notes


1) This paper is dedicated to Professor Shun-ichi Yamada on the occasion of his 70th birthday.
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