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# Enzymic Formation of β-Alkyl Glycosides by β-Galactosidase from *Aspergillus oryzae* and Its Application to the Synthesis of Chemically Unstable Cardiac Glycosides[1,2]

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β-Galactosidase obtained from *Aspergillus oryzae* was found to have strong transglycosylation activity. The glycosylation of water-soluble and insoluble alcohols was successfully performed under mild conditions (pH 5 at 4—37 °C) with this enzyme. The enzyme was also applied to the one-step synthesis of several cardiac glycosides that are difficult to obtain by ordinary chemical methods.

**Keywords**——enzymic transglycosylation; β-galactosidase; *Aspergillus oryzae*; cardiac glycoside; alkyl β-glycoside

Numerous glycosides of terpenoids and steroids can be found in nature. The physiological activity and bioavailability of these compounds frequently depend on the type or position of attached sugars. Thus, the creation of new drugs should be facilitated by the synthesis of the glycosides of naturally occurring genins and examination of their physiological properties. However, many problems still remain in the glycosylation of hydroxyl groups in terpenoids or steroids. For instance, in the Koenigs–Knorr method[3] frequently used for the synthesis of glycosides, genins with tertiary hydroxyl groups are liable to dehydration.[4] Furthermore, even if it is possible to synthesize a glycoside, decomposition during deacetylation of the sugar component may result in a very small yield of glycoside in the case of genins having a lactone ring or an aldehyde group unstable to alkali (*e.g.* cardiac glycosides of genins). On the other hand, enzymatic methods seem to be promising as a means of synthesizing such unstable glycosides because they can be carried out under mild conditions without decomposition of the starting materials or products. However, enzymatic methods[5a-c] so far reported have been performed in aqueous solution and are thus not applicable to the glycosylation of water-insoluble steroids or terpenoids. At our laboratory, several enzyme species from inexpensive industrial β-galactosidase have been found to be stable and to show great transglycosylation activity even in water–organic solvent media, and attempts were made to glycosylate water-insoluble genins by using the β-galactosidase[6a,b] obtained from *Aspergillus oryzae* (*Asp. oryzae*).

## Transglycosylation (TG) Reaction by β-Galactosidase from *Asp. oryzae* (I): TG Reaction in Water

Sugars were selected for the present TG reaction based on the measurement of the formation of methyl β-glycosides from phenyl β-glycosides, and β-galactosidase from *Asp. oryzae* was found to be suitable for the synthesis of β-galactosides and β-glucosides. The yields of alkyl β-galactosides and glucosides were examined with various alcohols and were measured by high performance liquid chromatography (HPLC) (Figs. 1 and 2) (Table I). When water-soluble alcohols were used to synthesize β-galactosides, alkyl β-galactosides were

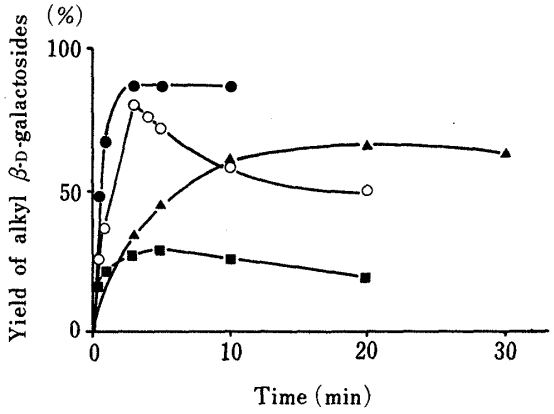



Fig. 1. Transgalactosylation to Alcohols in 0.1 M Phosphate Buffer (pH 5) at 35 °C

●, methyl-; ▲, 1-butyl-; ○, isopropyl-; ■, cyclohexyl-. HPLC conditions: column, 300 × 4 mm i.d. packed with μBondapak Carbohydrate analysis; mobile phase, $CH_3CN$–$H_2O$ (● (75:25), ▲ (85:15), ○ (80:20), ■ (85:15)); flow rate, ● 1.0 ml/min, ▲ 1.5 ml/min, ○ 2.0 ml/min, ■ 1.2 ml/min; detector, RI. Yields of alkyl β-galactosides were based on the phenyl β-galactoside.

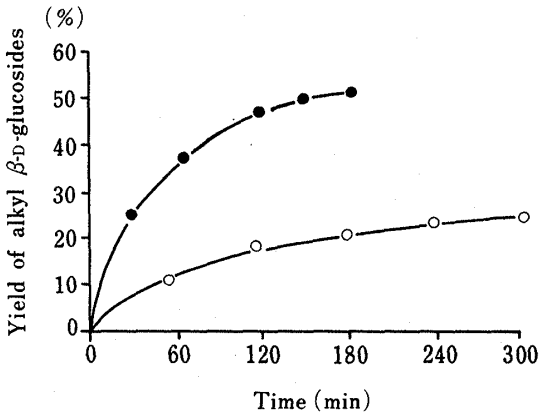



Fig. 2. Transglucosylation to Alcohols in 0.1 M Phosphate Buffer (pH 5) at 35 °C

●, methyl-; ○, cyclohexyl-. HPLC conditions: column, 300 × 4 mm i.d. packed with μBondapak Carbohydrate analysis; mobile phase, $CH_3CN$–$H_2O$ (● (75:25), ○ (85:15)); flow rate, ● 1.0 ml/min, ○ 1.2 ml/min; detector, RI. Yields of alkyl β-glucosides were based on the phenyl β-glucoside.

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

| Glycoside | Aglycone | Reaction time (min) | Yield[a] (%) | mp (°C) | NMR signal of anomeric proton (δ, Hz) | Formula | Calcd (Found) C | Calcd (Found) H |
|---|---|---|---|---|---|---|---|---|
| Galactoside | **1** Methyl | 5 | 82.9 | 177.5—177 | δ4.32 (d, $J$=8.1) | $C_7H_{14}O_6$ | 43.30 (43.43 | 7.27 7.65) |
| | **2** *n*-Butyl | 15 | 67.4 | 104—106 | δ4.39 (d, $J$=7.8) | $C_{10}H_{20}O_6$ | 50.84 (50.85 | 8.53 8.30) |
| | **3** Isopropyl | 3 | 74.5 | 114—116 | δ4.48 (d, $J$=7.8) | $C_9H_{18}O_6 \cdot 1/2H_2O$ | 46.75 (46.98 | 8.28 8.44) |
| | **4** Cyclohexyl | 5 | 25.0 | 130—132 | δ4.52 (d, $J$=8.1) | $C_{12}H_{22}O_6$ | 54.95 (55.10 | 8.45 8.70) |
| Glucoside | **5** Methyl | 150 | 48.8 | 104—106 | δ4.59 (d, $J$=8.1) | $C_7H_{14}O_6$ | 43.30 (43.11 | 7.27 7.45) |
| | **6** Cyclohexyl | 240 | 25.6 | 128—130 | δ4.38 (d, $J$=8.1) | $C_{12}H_{22}O_6 \cdot H_2O$ | 53.12 (53.13 | 8.54 8.51) |

ppm from internal DSS in $D_2O$. *a*) Isolated yield.

obtained in yields of 85—90% within a very short reaction time regardless of whether the alcohols were primary or secondary. The maximal yields were somewhat lower when 1-butanol or cyclohexanol was used. In the synthesis of β-glucosides, a long reaction time was required to achieve maximal yield.

**TG Reaction by β-Galactosidase from *Asp. oryzae* (II): TG Reaction in Aqueous–Organic Solvent Mixture**

Generally, an enzyme becomes unstable in aqueous–organic solvent mixtures and loses activity. This β-galactosidase from *Asp. oryzae* used in the present study was found to decrease in activity to 10 to 20% within 1 or 2 h in various aqueous organic solvents at 35 °C,

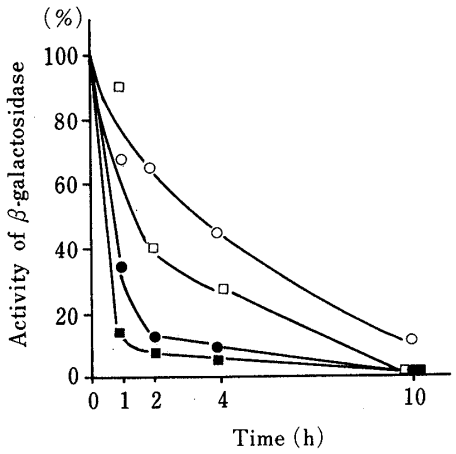



Fig. 3. Stability of β-Galactosidase in 50% Aqueous Organic Solvents at 35 °C

○, acetone; □, dioxane; ■, methanol; ●, acetonitrile.

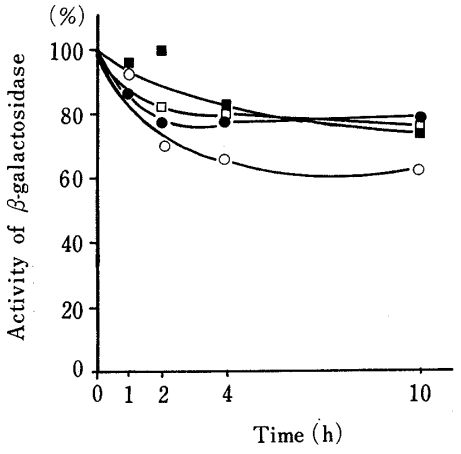



Fig. 4. Stability of β-Galactosidase in 50% Aqueous Organic Solvents at 4 °C

○, acetone; □, dioxane; ■, methanol; ●, acetonitrile.

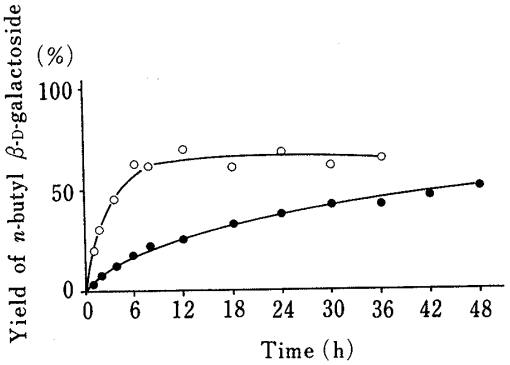



Fig. 5. Transgalactosylation to 1-Butanol at 4 °C in 50% Aqueous Organic Solvents

○, acetonitrile; ●, dioxane. HPLC conditions: column, 300 × 4 mm i.d. packed with μBondapak Carbohydrate analysis; mobile phase, $CH_3CN$–$H_2O$ (85 : 15); flow rate, 1.5 ml/min; detector, RI.

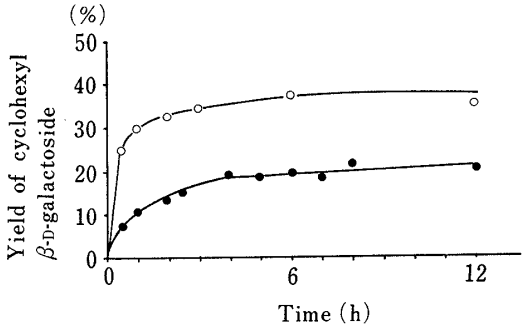



Fig. 6. Transgalactosylation to Cyclohexanol at 4 °C in 50% Aqueous Organic Solvents

○, acetonitrile; ●, dioxane. HPLC conditions: column, 300 × 4 mm i.d. packed with μBondapak Carbohydrate analysis; mobile phase, $CH_3CN$–$H_2O$ (85 : 15); flow rate, 1.2 ml/min; detector, RI.

and to maintain approximately 80% of its activity and sufficient stability for the TG reaction for 10 h at 4 °C (Figs. 3 and 4). Thus, 1-butanol and cyclohexanol were galactosylated in 50% aqueous organic solvents (acetonitrile and dioxane) (Figs. 5 and 6). The results showed that, although a somewhat prolonged reaction period was required, the products were obtained in higher yield than when the reaction took place in water, suggesting that the galactosylation of water-insoluble alcohols under such conditions may be practical.

**Synthesis of Cardiac Glycosides by the TG Reaction in 50% Acetonitrile**

Several cardiac genins unstable toward acids and bases were glycosylated in 50% aqueous acetonitrile. Approximately 1.28 mmol of a genin and 5 mmol of phenyl β-galactoside were dissolved in 40 ml of 62.5% acetonitrile. The enzyme (38 unit/mg) dissolved in 10 ml of 0.1 M phosphate buffer (pH 5) was then added to the solution, and the mixture was allowed to react (11400 units of enzyme at 20 °C for 20 min to synthesize the galactoside, and 40600 units of enzyme at 10 °C for 720 min to synthesize the glucosides). The reaction was terminated by boiling the mixture. The resulting glycosides were isolated in a conventional manner on Sephadex G-25 and by HPLC with a NUCLEOSIL $_{10}C_{18}$ column.

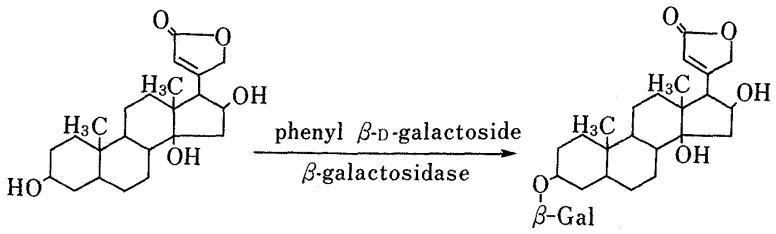
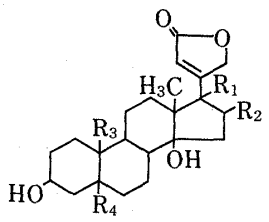



Chart 1



I: $R_1=R_4=H$, $R_2=OH$, $R_3=CH_3$ — gitoxigenin **(7)**
3-*O*-β-Gal **(7a)**
3-*O*-β-Glc **(7b)**

II: $R_1=R_2=R_4=H$, $R_3=CH_3$ — digitoxigenin **(8)**
3-*O*-β-Gal **(8a)**
3-*O*-β-Glc **(8b)**

III: $R_1=R_2=>O$, $R_3=CH_3$, $R_4=H$ — 16β,17β-epoxy-17α-digitoxigenin **(9)**
3-*O*-β-Gal **(9a)**

IV: $R_1=R_2=H$, $R_3=HCO$, $R_4=OH$ — strophanthidin **(10)**
3-*O*-β-Gal **(10a)**

Chart 2

TABLE II. $^{13}$C-NMR Chemical Shifts of Cardiac Glycosides

| Carbon | 7 | 7a | 7b | 8 | 8a | 8b | 9 | 9a | 10 | 10a |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 30.4 | 30.4 | 30.4 | 30.4 | 30.4 | 30.4 | 30.2 | 30.4 | 25.2 | 25.4 |
| 2 | 28.7 | 27.1 | 27.1 | 28.7 | 27.2 | 27.2 | 28.7 | 26.9 | 27.6 | 25.6 |
| 3 | 66.1 | 72.2 | 74.2 | 66.1 | 72.6 | 74.3 | 66.0 | 72.4 | 68.0 | 75.0 |
| 4 | 34.3 | 30.7 | 30.7 | 34.2 | 30.7 | 30.8 | 34.0 | 30.6 | 38.6 | 35.6 |
| 5 | 36.7 | 36.7 | 36.7 | 36.7 | 36.6 | 36.7 | 36.4 | 36.4 | 75.8 | 77.0 |
| 6 | 27.3 | 27.2 | 27.1 | 27.3 | 27.2 | 27.6 | 27.0 | 27.2 | 37.5 | 37.9 |
| 7 | 21.4 | 21.4 | 21.3 | 21.6 | 21.5 | 21.6 | 20.3 | 20.3 | 18.5 | 19.1 |
| 8 | 42.2 | 42.1 | 42.1 | 42.0 | 41.8 | 41.9 | 41.1 | 41.1 | 42.6 | 42.6 |
| 9 | 35.8 | 35.9 | 35.9 | 35.8 | 35.8 | 35.9 | 35.4 | 35.5 | 40.4 | 40.5 |
| 10 | 35.8 | 35.4 | 35.4 | 35.8 | 35.4 | 35.5 | 35.6 | 35.5 | 56.2 | 56.2 |
| 11 | 22.0 | 22.1 | 21.9 | 22.0 | 21.9 | 22.0 | 21.5 | 21.4 | 23.2 | 23.3 |
| 12 | 40.2 | 40.1 | 40.1 | 40.0 | 39.8 | 39.9 | 34.2 | 34.1 | 40.6 | 40.5 |
| 13 | 50.6 | 50.5 | 50.5 | 50.2 | 50.1 | 50.2 | 47.5 | 47.5 | 49.3 | 48.8 |
| 14 | 84.3 | 84.3 | 84.3 | 84.8 | 84.7 | 84.8 | 80.9 | 80.8 | 85.9 | 86.1 |
| 15 | 44.0 | 44.0 | 44.0 | 33.2 | 33.1 | 33.2 | 34.2 | 34.1 | 32.5 | 32.7 |
| 16 | 72.4 | 72.4 | 72.4 | 27.3 | 27.3 | 27.3 | 64.2 | 64.2 | 28.0 | 28.0 |
| 17 | 59.4 | 59.4 | 59.4 | 51.4 | 51.4 | 51.4 | 70.4 | 70.3 | 49.4 | 49.8 |
| 18 | 17.0 | 17.0 | 17.0 | 16.2 | 16.1 | 16.2 | 14.0 | 14.0 | 16.2 | 16.2 |
| 19 | 24.2 | 23.8 | 23.8 | 24.1 | 23.7 | 23.7 | 24.1 | 23.8 | 210.2 | 210.3 |
| 20 | 172.6 | 172.6 | 172.7 | 176.1 | 176.1 | 174.9 | 163.2 | 163.2 | 178.1 | 178.2 |
| 21 | 76.7 | 76.7 | 76.7 | 73.7 | 73.7 | 73.9 | 72.4 | 72.4 | 75.3 | 75.3 |
| 22 | 120.2 | 120.2 | 120.2 | 117.7 | 117.7 | 117.6 | 119.7 | 119.7 | 118.0 | 118.0 |
| 23 | 174.8 | 175.3 | 174.9 | 174.6 | 174.4 | 174.4 | 173.5 | 173.5 | 176.9 | 176.9 |
| 1′ | | 102.9 | 102.2 | | 103.8 | 103.0 | | 103.8 | | 102.7 |
| 2′ | | 72.8 | 75.4 | | 72.8 | 75.3 | | 72.7 | | 72.7 |
| 3′ | | 75.5 | 78.8 | | 75.5 | 78.6 | | 75.5 | | 76.0 |
| 4′ | | 70.2 | 71.1 | | 70.3 | 71.8 | | 70.3 | | 70.5 |
| 5′ | | 76.9 | 78.5 | | 76.9 | 78.3 | | 77.0 | | 76.9 |
| 6′ | | 62.5 | 62.9 | | 62.4 | 62.9 | | 62.5 | | 62.6 |

ppm from internal TMS in $C_5D_5N$.

The products were confirmed to be 3-$\beta$-*O*-glycosides by various instrumental analysis and by measuring the glycosidation shifts in the carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum[7] with respect to the aglycone[8] (Table II). In these reactions, no decomposition product derived from the glycosides or genins was found. This method should be useful for the glycosylation of aglycones that are unstable to acid or base. Glycosylation of sterically hindered hydroxyl groups is considered difficult in view of the unsuccessful results of glycosylation of *tert*-butanol and linalool.

## 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 JEOL GX-400 instrument. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Infrared (IR) spectra were recorded on a Shimadzu IR-27G or Hitachi 100-10 spectrophotometer. HPLC analysis were performed at room temperature on a Waters 6000A instrument with a $\mu$Bondapak carbohydrate analysis column (300 × 4 mm i.d.) and a refractive index (RI) detector (R-401) or a NUCLEOSIL $_{10}C_{18}$ column (600 × 8 mm i.d.) and an ultraviolet (UV) detector (UVIDEC 100-IV). The UV detector was set at 254 nm. The $\beta$-galactosidase preparations from *Asp. oryzae* were crude preparations with specific activities of 12 unit/mg[9] ($\beta$-glucosidase activity 0.2 unit/mg[10]) and 38 unit/mg ($\beta$-glucosidase activity 0.7 unit/mg). These curde preparations were kindly supplied by Kohjin Co., Ltd.[11] Phenyl $\beta$-D-galactoside and phenyl $\beta$-D-glucoside were prepared from the corresponding sugar in three steps according to the literature.[12a,b]

**Typical Homogeneous Transglycosylation: Preparation of Methyl $\beta$-D-Galactoside (1)**——Phenyl $\beta$-D-galactoside (600 mg, 2.34 mmol) and methanol (5.95 ml, 146.5 mmol) were dissolved in phosphate buffer (0.1 M, pH 5, 8.4 ml), and incubated at 35 °C. Phosphate buffer (0.1 M, pH 5, 10 ml) containing 2345 units of $\beta$-galactosidase was then added to the solution and the resulting mixture was allowed to react at 35 °C for 5 min. Sodium carbonate solution (0.1 M, 16 ml) was added to the solution to stop the enzymic reaction, and methanol was added to precipitate the enzyme.

The precipitate was removed by centrifugation. The supernatant was passed through a short cellulose column, then the eluate was concentrated and chromatographed on cellulose (1-BuOH sat. with $H_2O$) to give methyl $\beta$-galactoside (**1**) (390 mg).

**Measurement of the Rate of Glycoside Formation in Water**——Phenyl $\beta$-D-galactoside (25.6 mg, 0.1 mmol) and alcohol (6.25 mmol) were dissolved in phosphate buffer (0.1 M, pH 5, 0.5 ml), and incubated at 35 °C. Phosphate buffer (0.1 M, pH 5, 0.5 ml) containing 60 units of $\beta$-galactosidase was then added and the resulting mixture was allowed to react at 35 °C. Sodium carbonate solution (0.1 M, 0.5 ml) was added to the mixture to inactivate the enzyme.

Methanol was added to the solution to precipitate the enzyme. The precipitate was removed by centrifugation. The supernatant was concentrated and subjected to HPLC.

Methyl $\beta$-D-galactoside and methyl $\beta$-D-glucoside were eluted with $CH_3CN$–$H_2O$ (75 : 25) at a flow rate of 1.0 ml/min (internal standard : *myo*-inositol). 1-Butyl $\beta$-D-galactoside was eluted with $CH_3CN$–$H_2O$ (85 : 15) at a flow rate of 1.5 ml/min (internal standard: methyl $\beta$-D-glucoside).

Isopropyl $\beta$-D-galactoside was eluted with $CH_3CN$–$H_2O$ (80 : 20) at a flow rate of 2 ml/min (internal standard: *myo*-inositol). Cyclohexyl $\beta$-D-galactoside and cyclohexyl $\beta$-D-glucoside were eluted with $CH_3CN$–$H_2O$ (85 : 15) at a flow rate of 1.2 ml/min (internal standard: methyl $\beta$-D-glucoside).

**Stability of $\beta$-Galactosidase in 50% Aqueous Organic Solvents**——Hydrolytic activity towards *o*-nitrophenyl $\beta$-D-galactopyranoside (ONPG) was determined as a measure of the enzyme activity. $\beta$-Galactosidase (157 mg) was dissolved in phosphate buffer (0.1 M, pH 5, 20 ml) and organic solvent (20 ml) was added. The mixture was incubated in 35 °C. After 1, 2, 4, and 10 h, 0.1 ml of the solution was taken up and diluted to 50 ml with phosphate buffer (0.1 M, pH 5). The diluted solution (1 ml) and phosphate buffer (0.1 M, pH 5, 3 ml) containing 12 mg of ONPG were mixed, and the mixture was incubated at 30 °C. After incubation for 10 min, sodium carbonate solution (0.1 M, 2 ml) was added to stop the reaction, and the absorbance at 420 nm was read.

**Measurement of the Rate of Formation of Alkyl $\beta$-Glycoside in an Aqueous Organic Solvent**——Phenyl $\beta$-D-galactoside (25.6 mg, 0.1 mmol) and alcohol (6.25 mmol) were dissolved in phosphate buffer (0.1 M, pH 5, 0.4 ml) and organic solvent (0.5 ml), and the mixture was incubated at 4 °C. Phosphate buffer (0.1 M, pH 5, 0.1 ml) containing 60 units of enzyme was then added to the solution and the resulting mixture was allowed to react at 4 °C. Sodium carbonate solution (0.1 M, 1 ml) was added at the appropriate time to inactivate the enzyme, and methanol was added to precipitate the enzyme. The supernatant was concentrated and subjected to HPLC.

**3$\beta$-*O*-$\beta$-D-Galactopyranosyl-14,16$\beta$-dihydroxy-5$\beta$-card-20(22)-enolide (7a)**——Gitoxigenin (**7**) (0.5 g, 1.28 mmol) and phenyl $\beta$-D-galactoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 11400 units of the enzyme was added to the solution and the mixture was reacted 20 °C for

20 min. The reaction was terminated by boiling. The solution was filtered, then the filtrate was evaporated to remove acetonitrile. The starting material (**7**) was extracted with chloroform. The water layer was chromatographed on Sephadex G-25 (1-BuOH sat. with $H_2O$) and was purified by HPLC at room temperature on NUCLEOSIL $_{10}C_{18}$ (600 × 8 mm i.d.) with a UV detector set at 220 nm. The column was eluted with $CH_3CN$–$H_2O$ (30 : 70) at a flow rate of 3.0 ml/min to give a galactoside (**7a**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.37). IR (KBr): 3400 (OH), 1750, 1725 (CO of the lactone), 1620 (C=C) $cm^{-1}$. Optical rotatory dispersion (ORD) ($c$=0.100, methanol) $[\alpha]^{21.5}$ (nm): +126° (300). *Anal.* Calcd for $C_{29}H_{44}O_{10} \cdot 1/2H_2O$: C, 62.02; H, 8.08. Found: C, 61.54; H, 8.12. mp 226—228 °C. Yield 25.7%. Conversion ratio 2.0%.

**3β-*O*-β-D-Glucopyranosyl-14,16β-dihydroxy-5β-card-20(22)-enolide (7b)**——Gitoxigenin (**7**) (0.5 g, 1.28 mmol) and phenyl β-D-glucoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 40600 units of the enzyme was added to the solution and the mixture was reacted at 10 °C for 720 min. The reaction was terminated by boiling. The solution was filtered, then the filtrate was evaporated to remove acetonitrile. The starting material (**7**) was extracted with chloroform. The water layer was chromatographed on Sephadex G-25 (1-BuOH sat. with $H_2O$) and the product was purified by HPLC at room temperature on NUCLEOSIL $_{10}C_{18}$ (600 × 8 mm i.d.) with a UV detector set at 220 nm. The column was eluted with $CH_3CN$ : $H_2O$ (30 : 70) at a flow rate of 3.0 ml/min to give a glucoside (**7b**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.37). IR (KBr): 3450 (OH), 1760, 1720 (CO of the lactone), 1620 (C=C) $cm^{-1}$. ORD ($c$=0.100, methanol) $[\alpha]^{21.5}$ (nm): +110.3° (300). *Anal.* Calcd for $C_{29}H_{44}O_{10} \cdot H_2O$: C, 61.04; H, 8.12. Found: C, 61.00; H, 7.95. mp 217—219 °C. Yield 43.1%. Conversion ratio 2.0%.

**3β-*O*-β-D-Galactopyranosyl-14-hydroxy-5β-card-20(22)-enolide (8a)**——Digitoxigenin (**8**) (0.5 g, 1.28 mmol) and phenyl β-D-galactoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 11400 units of the enzyme was added to the solution and the mixture was reacted at 20 °C for 20 min. The reaction was terminated by boiling. The solution was filtered, then the filtrate was evaporated to remove acetonitrile. The starting material (**8**) was extracted with benzene. The water layer was chromatographed on Sephadex G-25 (1-BuOH sat. with $H_2O$) and the product was purified by HPLC to give a galactoside (**8a**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.23). IR (KBr): 3400 (OH), 1750, 1725 (CO of the lactone), 1620 (C=C) $cm^{-1}$. ORD ($c$= 0.101, methanol) $[\alpha]^{24}$ (nm): +119.2° (300). *Anal.* Calcd for $C_{29}H_{44}O_9 \cdot 1/2H_2O$: C, 63.83; H, 8.31. Found: C, 64.21; H, 8.53. mp 227—229 °C. Yield 38.4%. Conversion ratio 2.8%.

**3β-*O*-β-D-Glucopyranosyl-14-hydroxy-5β-card-20(22)-enolide (8b)**——Digitoxigenin (**8**) (0.5 g, 1.28 mmol) and phenyl β-D-glucoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 40600 units of the enzyme was added to the solution and the mixture was reacted at 10 °C for 720 min. The reaction was terminated by boiling. The solution was filtered, then the filtrate was evaporated to remove acetonitrile. The starting material (**8**) was extracted with benzene. The water layer was chromatographed on Sephadex G-25 (1-BuOH sat. with $H_2O$) and the product was purified by HPLC to give a glucoside (**8b**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.05). IR (KBr): 3400 (OH), 1750, 1720 (CO of the lactone), 1625 (C=C) $cm^{-1}$. ORD ($c$= 0.100, methanol) $[\alpha]^{24}$ (nm): +99.4° (300). *Anal.* Calcd for $C_{29}H_{44}O_9 \cdot H_2O$: C, 62.80; H, 8.36. Found: C, 62.68; H, 8.13. mp 231—233 °C. Yield 74.1%. Conversion ratio 2.2%.

**3β-*O*-β-D-Galactopyranosyl-14-hydroxy-16β,17β-epoxy-5β,17α-card-20(22)-enolide (9a)**——16β,17β-Epoxy-17α-digitoxigenin (**9**) (0.5 g, 1.287 mmol) and phenyl β-D-galactoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 11400 units of the enzyme was added to the solution and the mixture was reacted at 20 °C for 20 min. Acetone (60 ml) was added to the solution to stop the enzymic reaction and the precipitate formed was removed by centrifugation. The supernatant was evaporated to remove the organic solvents. The starting material (**9**) was extracted with benzene. The water layer was chromatographed on Sephadex G-25 (1-BuOH sat. with $H_2O$) to give a galactoside (**9a**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.39). IR (KBr): 3450 (OH), 2950, 1750, 1730 (CO of the lactone), 1620 (C=C) $cm^{-1}$. ORD ($c$=0.100, methanol) $[\alpha]^{21.5}$ (nm): +96.5° (300). *Anal.* Calcd for $C_{29}H_{42}C_{10}$: C, 63.26; H, 7.69. Found: C, 63.12; H, 7.93. dec. 243—246 °C. Yield 63.9%. Conversion ratio 2.3%.

**3β-*O*-β-D-Galactopyranosyl-5,14-dihydroxy-19-oxo-5β-card-20(22)-enolide (10a)**——Strophanthidin (**10**) (0.5 g, 1.24 mmol) and phenyl β-D-galactoside (1.28 g, 5 mmol) were dissolved in 62.5% acetonitrile (40 ml). Phosphate buffer (0.1 M, pH 5, 10 ml) containing 11400 units of the enzyme was added to the solution and the mixture was reacted at 20 °C for 20 min. Acetone (60 ml) was added to the solution to stop the enzymic reaction and the precipitate formed was removed by centrifugation. The supernatant was evaporated to remove the organic solvents. The starting material (**10**) was extracted with chloroform. The water layer was chromatographed on Sephadex LH-20 (methanol) to give a galactoside (**10a**). UV $\lambda_{max}^{MeOH}$ nm (log ε): 219 (4.48). IR (KBr): 3450 (OH), 2900, 1740, 1710 (CO of the lactone), 1620 (C=C) $cm^{-1}$. ORD ($c$=0.107, methanol) $[\alpha]^{21.5}$ (nm): +297° (300). *Anal.* Calcd for $C_{29}H_{42}O_{11} \cdot 2H_2O$: C, 57.80; H, 7.69. Found: C, 58.12; H, 7.51. mp 164—166 °C. Yield 40.0%. Conversion ratio 3.0%.

## References and Notes

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

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