Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular Catalysis B: Enzymatic 40 (2006) 8–15
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Synthesis of naturally occurring β-D-glucopyranoside based on enzymatic β-glycosidation

Hiroyuki Akita[a,*], Eiji Kawahara[a,b], Masashi Kishida[a,b], Keisuke Kato[a]

[a] *School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan*
[b] *Tsukuba Research Institute, Novartis Pharma K.K., 8 Ohkubo, Tsukuba-shi, Ibaraki 300-2611, Japan*

Received 12 January 2006; received in revised form 24 January 2006; accepted 25 January 2006
Available online 28 February 2006

Abstract

For the purpose of synthesis of naturally occurring β-D-glucopyranoside, direct β-glycosidation for seven kinds of the functionalized primary alcohol in the presence of D-glucose using native or immobilized β-glucosidase (EC 3.2.1.21) from almonds under equilibrium condition was carried out. The utilization of high concentration of the alcohol acceptor, 3-methyl-2-buten-1-ol (**3**) or 2-methyl-2-propen-1-ol (**4**) using the immobilized enzyme gave the corresponding β-D-glucopyranoside (**21**, natural product) or (**22**) in 65 or 51% yield, respectively. On the other hand, the utilization of 4-equivalents of the functionalized alcohols (**5**–**9**) using the immobilized enzyme in 90% *tert*-butanol/H_2O solution afforded the naturally occurring β-D-glucopyranosides (**23**–**27**), respectively, in moderate yield. Among them, five kinds of β-D-glucopyranosides (**21**, **22**, **24**, **25**, and **27**) were converted into the cyanoglucosides (rhodiocyanoside A (**28**), osmaronin (**29**)) and other naturally occurring β-D-glucopyranosides (**30**–**32**), respectively.

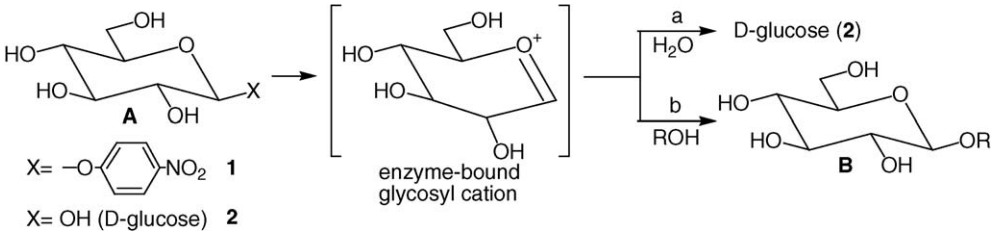
Keywords: β-glucosidase; β-glycosidation; β-D-glucopyranoside; Natural product synthesis

1. Introduction

There are many β-D-glucopyranosides possessing a primary alcohol moiety as an aglycone part in nature. The development of stereoselective methods for the synthesis of glycosidic linkages presents a considerable challenge to synthetic chemists [1,2]. Although well-developed chemical synthesis of the glycosidic structure is increasingly being established, several steps of selective protection, activation and coupling are necessary. This problem in chemical synthesis has promoted the development of enzymatic approaches. Lipase-catalyzed synthesis of acyl sugar is reported [3], whereas much less is known about glycosidase-catalyzed synthesis of alkyl glycosides [4]. Glycosidases are responsible for formation of the glycosidic linkage and are increasingly being used in carbohydrate synthesis. The β-glucosidase catalyzes the hydrolytic cleavage of the β-glucosidic bond in the substrate **A** such as *p*-nitrophenyl-β-D-glucopyranoside (**1**) to give the glycosyl cation intermediate, which is trapped by water or a nucleophile such as an alcohol to afford D-glucose (**2**) (Scheme 1, path a) or a new β-D-glucopyranoside **B** (Scheme 1, path b) under kinetic condition, respectively. On the other hand, β-glucosidases may be used for the direct formation of β-D-glucopyranoside **B** from alcohol in the presence of D-glucose (**2**) via an oxonium ion intermediate (enzyme bound glycosyl cation) under equilibrium condition. In the case where the alcohol is cheap and readily available, the use of high concentration of the alcohol acceptor in order to favor formation of β-D-glucopyranoside **B** over the starting D-glucose (**2**) is an excellent procedure for direct β-glycosidation [5]. Moreover, the use of 90% *tert*-butanol (*tert*-BuOH:H_2O = 90:10 (v:v)) as an organic co-solvent is reported to be useful in the direct β-glycosidation because of decrease of the water concentration and increase of solubility of the alcohol [6]. We are attracted to this transglycosylation reaction since alcohols as the glycosyl acceptor are better bound at the active site than water. Phenylpropenoid glucosides are reported to be pharmacologically active substances such as antioxidants, neurostimulants and antihypertensive ingredient [7–11]. We now report the direct synthesis of naturally occurring β-D-glucopyranoside **B** including phenylpropenoid glucosides from the starting func-

* Corresponding author. Tel.: +81 47 472 1805; fax: +81 47 476 6195.
E-mail address: akita@phar.toho-u.ac.jp (H. Akita).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.01.031



Scheme 1. Formation of glucose (**2**) or β-D-glucopyranoside (**B**) from β-D-glucopyranoside (**A**) via glycosy cation.

tionalized primary alcohol such as cinnamyl alcohol congeners using the native or immobilized β-glucosidase (EC 3.2.1.21) from almonds.

2. Material and methods

2.1. Analytical methods

^{1}H and ^{13}C NMR spectra were recorded by a JEOL EX 400 spectrometer (Tokyo, Japan). Spectra were taken with 5–10% (w/v) solution in $CDCl_3$ with Me_4Si as an internal reference. Melting points were determined on a Yanaco MP-3S micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. The FAB mass spectra were obtained with a JEOL JMS-AX 500 (matrix; *m*-nitrobenzyl alcohol) spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

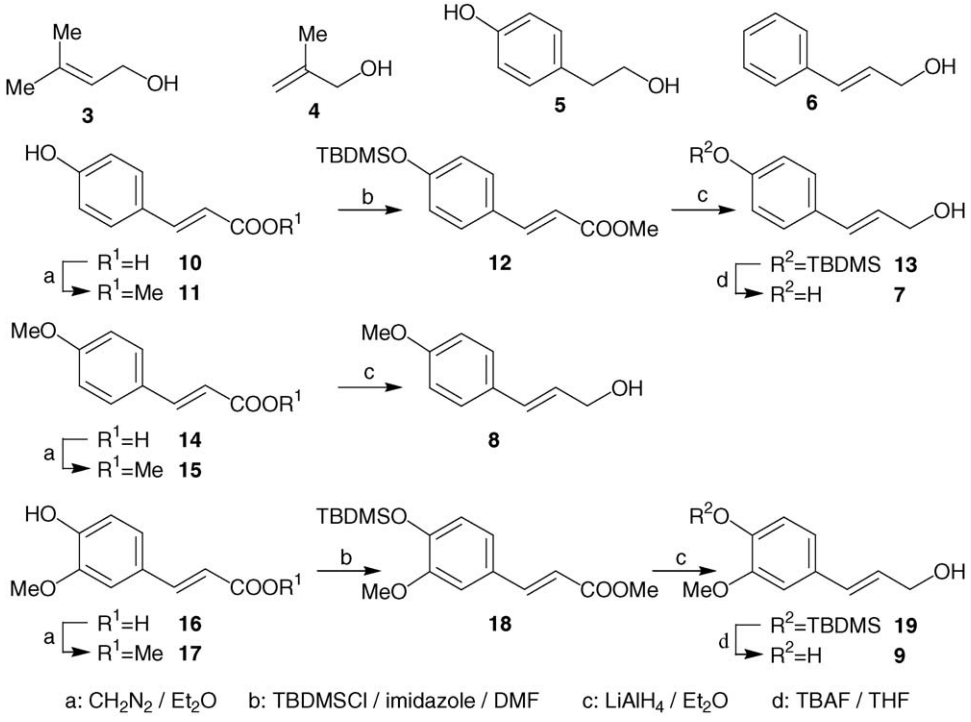
2.2. Materials

2.2.1. Enzymes

The β-D-glucosidase (EC 3.2.1.21) from almonds was purchased from Sigma Chemical Co. (G-0395, 2.5–3.6 units/mg). One unit of the enzyme activity was defined as the amount of enzyme catalyzing the production of 1 μmol of D-glucose from *p*-nitrophenyl β-D-glucopyranoside per min.

2.2.2. Glycosyl acceptors

1. The following glycosyl acceptors were commercially available. 3-Methyl-2-buten-1-ol (**3**), 2-methyl-2-propen-1-ol (**4**), 4-hydroxyphenethyl alcohol (**5**), cinnamyl alcohol (**6**) were purchased from Sigma–Aldrich Co., Ltd.
2. The following glycosyl acceptors, 4-hydroxycinnamyl alcohol (**7**), 4-methoxycinnamyl alcohol (**8**) and 4-hydroxy-3-methoxycinnamyl alcohol (**9**) were synthesized as shown in Scheme 2. Treatment of the commercially available substrates **10**, **14** and **16** with CH_2N_2 gave the corresponding



Scheme 2. Glycosyl acceptors (alcohols) and synthesis of cinnamyl alcohol congners.

methyl esters **11**, **15** and **17**, respectively. Protection of the phenol group of **11** and **17** followed by $LiAlH_4$ reduction gave the alcohols **12** and **18**, which were subjected to deprotection to provide the cinnamyl alcohol congeners **7** and **9**, respectively. $LiAlH_4$ reduction of **15** gave the cinnamyl alcohol congener **8**.

*2.2.2.1. 4-Hydroxycinnamyl alcohol (**7**).* (i) Treatment of 4-hydroxycinnamic acid (**10**, 11.0 g, 67.1 mmol) with CH_2N_2–Et_2O solution gave a crude methyl ester, which was purified by chromatography on silica gel (120 g, *n*-hexane:AcOEt (5:1)) to afford colorless crystals (**11**, 11.0 g, 92%). Recrystallization of **11** from *n*-hexane:AcOEt gave colorless needles (**11**). **11**: mp 130–135 °C; IR (KBr): 3374, 2921, 1685, 1593, 1282, 1198 cm^{-1}; ^1H NMR: δ 3.79 (3H, s), 5.96 (1H, s), 6.28 (1H, d, J = 16.0 Hz), 6.84 (2H, d, J = 8.6 Hz), 7.40 (2H, d, J = 8.6 Hz), 7.63 (1H, d, J = 16.0 Hz). Anal. calcd. for $C_{10}H_{10}O_3$: C, 67.41; H, 5.66. Found: C, 67.13; H, 5.67. (ii) To a solution of **11** (11.0 g, 61.8 mmol) in DMF (30 ml) was added *tert*-butyldimethylsilyl chloride (TBDMSCl, 14.0 g, 92.7 mmol) and imidazole (12.6 g, 183.5 mmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (180 g, *n*-hexane:AcOEt (5:1)) to afford a colorless oil (**12**, 16.03 g, 89%). **12**: IR (KBr): 2932, 1715, 1598, 1264, 1167 cm^{-1}; ^1H NMR: δ 0.20 (6H, s), 0.96 (9H, s), 3.77 (3H, s), 6.28 (1H, d, J = 16.0 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.39 (2H, d, J = 8.8 Hz), 7.62 (1H, d, J = 16.0 Hz). FAB-MS *m/z*: 293 (M^+ + H). (iii) To a solution of **12** (16.0 g, 54.8 mmol) in Et_2O (30 ml) was added $LiAlH_4$ (2.3 g, 60.6 mmol) at 0 °C, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with water and filtered with the aid of Celite. The filtrate was extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (145 g, *n*-hexane:AcOEt (5:1)) to afford a colorless oil (**13**, 12.16 g, 84%). **13**: IR (KBr): 3247, 2933, 1508, 1263, 914 cm^{-1}; ^1H NMR: δ 0.20 (6H, s), 0.98 (9H, s), 4.26 (2H, d, J = 6.0 Hz), 6.21 (1H, dt, J = 6.0, 16.0 Hz), 6.52 (1H, d, J = 16.0 Hz), 6.78 (2H, d, J = 8.8 Hz), 7.25 (1H, d, J = 8.8 Hz). EI-MS *m/z*: 264 (M^+). (iv) To a solution of **13** (7.19 g, 27.2 mmol) in THF (10 ml) was added 1 mol tetrabutylammonium fluoride (TBAF)-THF solution (27.2 ml, 27.2 mmol) at 0 °C, and the mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (45 g, *n*-hexane:AcOEt (5:1)) to afford colorless plates (**7**, 3.8 g, 93%). Recrystallization of **7** from *n*-hexane:AcOEt gave colorless plates (**7**). **7**: mp 114–116 °C; **7**: IR (KBr): 3283, 2921, 1507, 1252 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 2.88 (1H, s), 3.72 (1H, s), 4.28 (2H, br), 6.19 (1H, dt, J = 5.5, 15.8 Hz), 6.50 (1H, d, J = 15.8 Hz), 6.78 (2H, d, J = 8.4 Hz), 7.26 (1H, d, J = 8.4 Hz). Anal. calcd. for $C_9H_{10}O_2$: C, 71.98; H, 6.71. Found: C, 71.93; H, 6.79.

*2.2.2.2. 4-Methoxycinnamyl alcohol (**8**).* (i) Treatment of 4-methoxycinnamic acid (**14**, 10.0 g, 56.2 mmol) with CH_2N_2–Et_2O solution gave a crude methyl ester, which was purified by chromatography on silica gel (120 g, *n*-hexane:AcOEt (10:1)) to afford colorless plates (**15**, 10.41 g, 96%). Recrystallization of **15** from *n*-hexane:AcOEt gave colorless plates (**15**). **15**: mp 83–88 °C; IR (KBr): 1714, 1638, 1293, 1171, 1016 cm^{-1}; ^1H NMR: δ 3.78 (3H, s), 3.82 (3H, s), 6.29 (1H, d, J = 16.0 Hz), 6.88 (2H, d, J = 8.6 Hz), 7.45 (2H, d, J = 8.6 Hz), 7.63 (1H, d, J = 16.0 Hz). Anal. calcd. for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.47; H, 6.28. (ii) To a solution of **15** (10.0 g, 52.1 mmol) in Et_2O (100 ml) was added $LiAlH_4$ (2.1 g, 55.3 mmol) at 0 °C, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with water and filtered with the aid of Celite. The filtrate was extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (100 g, *n*-hexane:AcOEt (7:1)) to afford colorless plates (**8**, 7.56 g, 89%). **8**: mp 130–132 °C; IR (KBr): 3345, 2924, 1604, 1243, 1018 cm^{-1}; ^1H NMR(CD_3OD): δ 3.76 (3H, s), 4.18 (2H, d, J = 5.9 Hz), 6.20 (1H, dt, J = 5.9, 16.0 Hz), 6.48 (1H, d, J = 16.0 Hz), 6.85 (2H, d, J = 8.8 Hz), 7.31 (2H, d, J = 8.8 Hz). Anal. calcd. for $C_{10}H_{12}O_2$: C, 73.15; H, 7.37. Found: C, 72.88; H, 7.35.

*2.2.2.3. 4-Hydroxy-3-methoxycinnamyl alcohol (**9**).* (i) Treatment of 4-hydroxy-3-methoxycinnamic acid (**16**, 10.0 g, 51.5 mmol) with CH_2N_2-Et_2O solution gave a crude methyl ester, which was purified by chromatography on silica gel (120 g, *n*-hexane:AcOEt (7:1)) to afford a colorless oil (**17**, 10.53 g, 98%). **17**: IR (KBr): 3399, 2950, 1701, 1636, 1591, 1270, 1171 cm^{-1}; ^1H NMR: δ 3.80 (3H, s), 3.93 (3H, s), 6.29 (1H, d, J = 16.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 7.03 (1H, d, J = 1.8 Hz), 7.08 (1H, dd, J = 1.8, 8.0 Hz), 7.62 (1H, d, J = 16.0 Hz). FAB-MS *m/z*: 209 (M^+ + H). (ii) To a solution of **17** (10.0 g, 48.1 mmol) in DMF (40 ml) was added TBDMSCl (14.8 g, 98 mmol) and imidazole (9.8 g, 144.3 mmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (150 g, *n*-hexane:AcOEt (5:1)) to afford a colorless oil (**18**, 15.19 g, 98%). **18**: IR (KBr): 2925, 1711, 1511, 1285, 1169, 1030, 915 cm^{-1}; ^1H NMR: δ 0.15 (6H, s), 0.97 (9H, s), 3.77 (3H, s), 3.81 (3H, s), 6.28 (1H, d, J = 16.0 Hz), 6.82 (1H, d, J = 8.8 Hz), 6.99–7.12 (2H, m), 7.60 (1H, d, J = 16.0 Hz). FAB-MS *m/z*: 323 (M^+ + H). (iii) To a solution of **18** (15.0 g, 46.4 mmol) in Et_2O (20 ml) was added $LiAlH_4$ (1.8 g, 47.4 mmol) at 0 °C, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with water and filtered with the aid of Celite. The filtrate was extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent

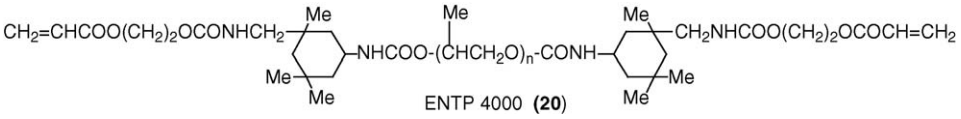


Fig. 1. Structure of photo-crosslinkable resin prepolymer.

gave a crude product, which was purified by chromatography on silica gel (130 g, *n*-hexane:AcOEt (5:1)) to afford a colorless oil (**19**, 9.75 g, 71%). **19**: IR (KBr): 3355, 2936, 1512, 1284, 915 cm^{-1}; ^1H NMR: δ 0.63 (6H, s), 1.48 (9H, s), 4.20 (3H, s), 4.69 (2H, d, J = 5.9 Hz), 6.64 (1H, dt, J = 5.9, 16.0 Hz), 6.93 (1H, d, J = 16.0 Hz), 7.23 (1H, d, J = 8.0 Hz), 7.27 (1H, dd, J = 2.0, 8.0 Hz), 7.32 (1H, d, J = 2.0 Hz). FAB-MS *m/z*: 295 (M^+ + H). (iv) To a solution of **19** (9.75 g, 33.2 mmol) in THF (10 ml) was added 1 mol TBAF-THF solution (36.9 ml, 36.9 mmol) at 0 °C, and the mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was purified by chromatography on silica gel (65 g, *n*-hexane:AcOEt (5:1)) to afford colorless plates (**9**, 4.78 g, 80%). Recrystallization of **9** from *n*-hexane:Et_2O gave colorless plates (**9**). **9**: mp 68–73 °C; IR (KBr): 3465, 3246, 2924, 1516, 1393, 1257, 1009 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 3.79 (1H, br.s), 3.85 (3H, s), 4.19 (2H, d, J = 5.2 Hz), 6.22 (1H, dt, J = 5.2, 16.0 Hz), 6.54 (1H, d, J = 16.0 Hz), 6.76 (1H, d, J = 8.4 Hz), 6.86 (1H, dd, J = 2.0, 8.4 Hz), 7.04 (1H, d, J = 2.0 Hz), 7.59 (1H, br.s). Anal. calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.46; H, 6.78.

2.3. Immobilization of β-D-glucosidase using prepolymer

Immobilization of β-D-glucosidase from almonds on photo-crosslinkable resin prepolymer (ENTP-4000) was carried out by the following procedure. One gram of ENTP-4000 (**20**) [12] was mixed with 10 mg of a photosensitizer, benzoin ethyl ether and 110 mg of β-D-glucosidase from almonds (3.4 units/mg). The mixture was layered on a sheet of transparent polyester film (thickness, ca. 0.5 mm). The layer was covered with transparent thin film and then illuminated with chemical lamps (wavelength range, 300–400 nm) for 3 min. The gel film thus obtained was cut into small pieces (0.5 mm × 5 mm × 5 mm) and used for bioconversion reaction (Fig. 1).

3. Results and discussion

3.1. Enzymatic transglycosylation

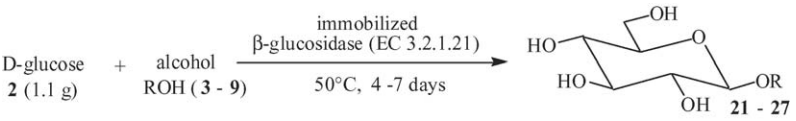
A general procedure of the synthesis of β-D-glucopyranoside **B** using alcohol was carried out as follows. A mixture of D-glucose (**2**, 1.1 g), native β-glucosidase (ca. 370 units) or the above-mentioned immobilized β-glucosidase (ca. 1.1 g, corresponding to ca. 370 units), alcohol (18 ml) and H_2O (2 ml) was incubated for 4 d at 50 °C (method I). A mixture of D-glucose (**2**, 1.1 g), the above-mentioned immobilized β-glucosidase (ca. 1.1 g, corresponding to ca. 370 units), alcohol (4 equivalents) in 90% (v/v) *tert*-butanol (27 ml) and H_2O (3 ml) was incubated for 7 d at 50 °C (method II). The results are shown in Table 1. The structure of the synthesized products (**21**–**27**) was confirmed by elemental analysis, spectroscopic analysis and a comparison with the reported data. As the chemical yield (Table 1, entry 2) of β-D-glucopyranoside (**21**) using the immobilized β-glucosidase appeared to be better than that (Table 1, entry 1) using native enzyme, the immobilized β-glucosidase was used as shown below.

3.1.1. Synthesis of β-D-glucopyranoside by method I

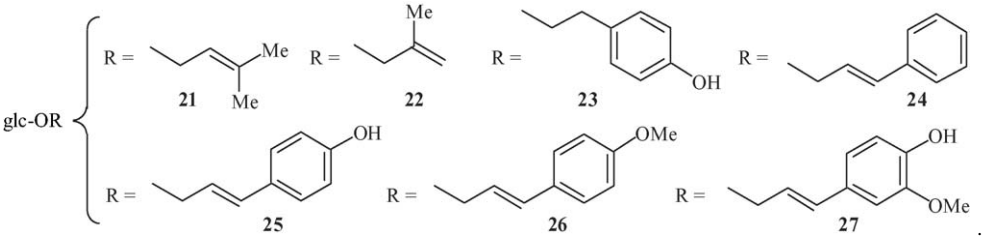
*3.1.1.1. 3-Methyl-2-butenyl β-D-glucopyranoside (**21**).* (i) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 3-methyl-2-buten-1-ol (**3**, 15.3 g, 177.2 mmol), water (2 ml), and the native β-glucosidase (ca. 370 units) was incubated for 4 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (35 g) to give 3-methyl-2-buten-1-ol (12.0 g, 78% recovery) from the $CHCl_3$ eluent and β-D-glucopyranoside (**21**, 0.867 g, 57% yield) as a colorless solid from the $CHCl_3$/MeOH = 10:1 eluent. The NMR (^1H and ^{13}C NMR) data of β-D-glucopyranoside (**21**) were identical with those of the reported β-D-glucopyranoside (**21**) [13]. **21**: mp 78–81 °C; $[\alpha]_D^{26}$ −40.8 (c = 0.54, MeOH); IR (KBr): 3346, 2882, 1073, 1025 cm^{-1}, ^1H NMR (pyridine-d_5): δ 1.54 (3H, s), 1.60 (3H, s), 3.94–3.97 (1H, m), 4.06 (1H, t, J = 8 Hz), 4.21–4.29 (2H, m), 4.38 (1H, d, J = 11.7 Hz), 4.36–4.40 (1H, m), 4.59 (1H, d, J = 11.7 Hz), 4.54–4.55 (1H, m), 4.90 (1H, d, J = 7.3 Hz), 5.52 (1H, t, J = 6.3 Hz); ^{13}C NMR (pyridine-d_5): δ 135.9, 121.9, 103.5, 78.6, 78.5, 75.2, 71.7, 65.7, 62.8, 25.6, 17.9. Anal. calcd. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 52.78; H, 8.24. (ii) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 3-methyl-2-buten-1-ol (**3**, 15.3 g, 177.2 mmol), water (2 ml), and the mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) was incubated for 4 d at 50 °C. The reaction mixture was filtered off and the filtrate was worked up in the same way as for (i) to give 3-methyl-2-buten-1-ol (12.0 g, 78% recovery) and β-D-glucopyranoside (**21**, 0.983 g, 65% yield). (iii) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 3-methyl-2-buten-1-ol (**3**, 15.3 g, 177.2 mmol), water (2 ml), and the recovered immobilized β-glucosidase (1.1 g, ca. 370 units) from the experiment (ii) was incubated for 4 d at 50 °C. The reaction mixture was worked up in the same way as for (ii) to give 3-methyl-2-buten-1-ol (11.0 g, 72% recovery) and β-D-glucopyranoside (**21**, 0.450 g, 30% yield).

*3.1.1.2. 2-Methyl-2-propenyl β-D-glucopyranoside (**22**).* A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 2-methyl-2-propen-1-ol (**4**, 15.4 g, 213.9 mmol), water (2 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) was incubated for 4 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography

Table 1
Synthesis of β-D-glucopyranosides using seven kinds of alcohols



Entry	ROH (eq)	Method (I or II)	Time (d)	glc-OR (yield, %)
1[a]	**3** (29)	I	4	**21** (57)
2	**3** (29)	I	4	**21** (65)
3[b]	**3** (29)	I	4	**21** (30)
4	**4** (35)	I	4	**22**(51)
5	**5** (4)	II	7	**23** (11)
6[b]	**5** (4)	II	7	**23** (9)
7	**6** (4)	II	7	**24** (8)
8[b]	**6** (4)	II	7	**24** (6)
9	**7** (4)	II	7	**25** (11)
10	**8** (4)	II	7	**26** (8)
11	**9** (4)	II	7	**27** (8)



[a] Native β-glucosidase was used.
[b] The recovered immobilized β-glucosidase was used.

on silica gel (35 g) to give 2-methyl-2-propen-1-ol (8.2 g, 53% recovery) from the $CHCl_3$ eluent and β-D-glucopyranoside (**22**, 0.732 g, 51% yield) as a colorless solid from the $CHCl_3$/MeOH = 10:1 eluent. **22**: mp 123–125 °C; $[\alpha]_D^{25}$ −51.7 (c = 0.24, MeOH); IR (KBr): 3434, 1648, 1455, 1380, 1082 cm^{-1}, ^1H NMR (D_2O): δ 1.63 (3H, s), 3.14–3.47 (4H, m),3.58 (1H, dd, J = 5.9, 12.2 Hz), 3.77 (1H, dd, J = 1.0, 12.2 Hz), 4.08 (1H, d, J = 12.7 Hz), 4.15 (1H, d, J = 12.7 Hz), 4.33 (1H, d, J = 7.8 Hz), 4.88 (1H, s), 4.93 (1H, s); ^{13}C NMR (D_2O): δ 142.3, 114.4, 101.7, 76.6, 76.6, 73.9, 73.9, 70.5, 61.5, 19.5. Anal. calcd. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.75. Found: C, 51.12; H, 7.79.

3.1.2. Synthesis of β-D-glucopyranoside by method II

*3.1.2.1. 4-Hydroxyphenethyl β-D-glucopyranoside (Salidroside) (**23**).* (i) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 4-hydroxyphenethyl alcohol (**5**, 3.36 g, 24.3 mmol), water (3 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (150 g) to give 4-hydroxyphenethyl alcohol (**5**, 3.02 g, 90% recovery) from the $CHCl_3$/MeOH = 20:1 eluent and β-D-glucopyranoside (**23**, 0.202 g, 11% yield) as a colorless solid from the $CHCl_3$/MeOH = 9:1 eluent. The NMR (^1H and ^{13}C NMR) data of β-D-glucopyranoside (**23**) were identical with those of the reported β-D-glucopyranoside (**23**) [14–18]. **23**: mp 159–160 °C; $[\alpha]_D^{26}$ −28.4 (c = 0.5, MeOH); IR (KBr): 3324, 2956, 1520, 1075, 1060 cm^{-1}, ^1H NMR (CD_3OD): δ 2.81–2.85 (2H, m), 3.19 (1H, t, J = 7.8 Hz), 3.24–3.38 (3H, m), 3.65–3.73 (2H, m), 3.87 (1H, dd, J = 2.0, 12.2 Hz), 4.00–4.06 (1H, m), 4.29 (1H, d, J = 7.8 Hz), 6.69 (2H, d, J = 8.8 Hz), 7.06 (2H, d, J = 8.8 Hz); ^{13}C NMR (CD_3OD): δ 156.8, 130.9, 130.9, 130.7, 116.1, 116.1, 104.4, 78.1, 77.9, 75.1, 72.1, 71.6, 62.7, 36.4. Anal. calcd. for $C_{14}H_{20}O_7$: C, 55.91; H, 6.71. Found: C, 55.63; H, 6.77. (ii) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 4-hydroxyphenethyl alcohol (**5**, 3.36 g, 24.3 mmol), water (3 ml), and the recovered immobilized β-glucosidase (1.1 g, ca. 370 units) from the experiment (i) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was worked up in the same way as for (i) to give 4-hydroxyphenethyl alcohol (**5**, 3.1 g, 95% recovery) and β-D-glucopyranoside (**23**, 0.162 g, 9% yield).

*3.1.2.2. Cinnamyl β-D-glucopyranoside (Rosin) (**24**).* (i) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), cinnamyl alcohol (**6**, 3.66 g, 24.4 mmol), water (3 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (150 g) to give cinnamyl alcohol (**6**, 3.0 g, 92% recovery) from the $CHCl_3$/MeOH = 20:1 eluent and β-D-glucopyranoside (**24**, 0.145 g, 8% yield) as colorless solid from the $CHCl_3$/MeOH = 9:1 eluent. The NMR (^1H and ^{13}C NMR) data of β-D-glucopyranoside (**24**) were identical with those of the reported β-D-glucopyranoside (**24**) [7,8]. **24**: mp 114–116 °C; $[\alpha]_D^{28}$ −48.8 (c = 0.3, MeOH); IR (KBr): 3398, 1652, 1564, 1075 cm^{-1}, ^1H NMR (CD_3OD): δ 3.20–3.38 (4H, m), 3.65–3.70

(1H, m), 3.88 (1H, dd, J = 5.0, 11.6 Hz), 4.32 (1H, ddd, J = 1.5, 6.5, 12.6 Hz), 4.36 (1H, d, J = 7.6 Hz), 4.53 (1H, ddd, J = 1.5, 6.0, 12.6 Hz), 6.37 (1H, dt, J = 15.6, 6.0 Hz), 6.68 (1H, d, J = 15.6 Hz), 7.19–7.23 (1H, m), 7.27–7.32 (2H, m), 7.40–7.43 (2H, m); ^{13}C NMR (CD_3OD): δ 138.0, 133.5, 129.3, 128.4, 127.3, 126.5, 103.1, 77.9, 77.8, 74.9, 71.5, 70.6, 62.6; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{15}H_{20}O_6$: 297.1338 (M^+ + H); found, 297.1333. (ii) A mixture of D-glucose **2** (1.1 g, 6.1 mmol), cinnamyl alcohol (**5**, 3.66 g, 24.4 mmol), water (3 ml), and the recovered immobilized β-glucosidase (1.1 g, ca. 370 units) from the experiment (i) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was worked up in the same way as for (i) to give cinnamyl alcohol (**5**, 3.1 g, 95% recovery) and β-D-glucopyranoside (**24**, 0.109 g, 6% yield).

*3.1.2.3. 4-Hydroxycinnamyl β-D-glucopyranoside (Sachaliside 1, Trandirin) (**25**).* A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 4-hydroxycinnamyl alcohol (**7**, 3.66 g, 24.4 mmol), water (3 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (150 g) to give 4-hydroxycinnamyl alcohol (**7**, 3.3 g, 90% recovery) from the $CHCl_3$/MeOH = 20:1 eluent and β-D-glucopyranoside (**25**, 0.217 g, 11% yield) as a colorless solid from the $CHCl_3$/MeOH = 9:1 eluent. The NMR (^{1}H and ^{13}C NMR) data of β-D-glucopyranoside (**25**) were identical with those of the reported β-D-glucopyranoside (**25**) [9,10]. **25**: mp 118–123 °C; $[\alpha]_D^{22}$ −62.2 (c = 0.6, MeOH); IR (KBr): 3299, 2929, 1609, 1515, 1249, 1170, 1083, 841 cm^{-1}, ^{1}H NMR (pyridine-d_5): δ 3.94–4.00 (1H, m), 4.08–4.14 (1H, m), 4.24–4.32 (2H, m), 4.41 (1H, dd, J = 5.0, 11.6 Hz), 4.47 (1H, dd, J = 6.0, 12.6 Hz), 4.58 (1H, dd, J = 1.5, 11.6 Hz), 4.75 (1H, dd, J = 6.0, 12.6 Hz), 4.99 (2H, d, J = 7.6 Hz), 6.36 (1H, dt, J = 6.0, 15.6 Hz), 6.73 (1H, d, J = 15.6 Hz), 7.15 (2H, d, J = 8.6 Hz), 7.40 (2H, d, J = 8.6 Hz); ^{13}C NMR (pyridine-d_5): δ 158.9, 132.6, 128.6, 128.4, 116.5, 103.9, 78.59, 78.55, 75.3, 71.7, 70.1, 62.8; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{15}H_{20}O_7$: 313.1287 (M^+ + H); found, 313.1288.

*3.1.2.4. 4-Methoxycinnamyl β-D-glucopyranoside (Vimalin) (**26**).* A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 4-methoxycinnamyl alcohol (**8**, 4.0 g, 24.4 mmol), water (3 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (150 g) to give 4-methoxycinnamyl alcohol (**8**, 3.82 g, 95% recovery) from the $CHCl_3$/MeOH = 30:1 eluent and β-D-glucopyranoside (**26**, 0.163 g, 8% yield) as a colorless solid from the $CHCl_3$/MeOH = 10:1 eluent. The NMR (^{1}H and ^{13}C NMR) data of β-D-glucopyranoside (**26**) were identical with those of the reported β-D-glucopyranoside (**26**) [9]. **26**: mp 111–123 °C; $[\alpha]_D^{22}$ −54.7 (c = 1.4, MeOH); IR (KBr): 3350, 2938, 1605, 1514, 1245, 1169, 1083, 1022, 968, 781 cm^{-1}, ^{1}H NMR (pyridine-d_5): δ 3.66 (3H, s), 3.95–4.02 (1H, m), 4.11–4.15 (1H, m), 4.25–4.32 (2H, m), 4.41–4.50 (2H, m), 4.59 (1H, dd, J = 2.0, 11.6 Hz), 4.76 (1H, ddd, J = 1.5, 5.6, 12.6 Hz), 4.99 (1H, d, J = 8.0 Hz), 6.38 (1H, dt, J = 16.1, 6.0 Hz), 6.74 (1H, d, J = 16.1 Hz), 6.95 (1H, d, J = 8.6 Hz), 7.38 (1H, d, J = 8.6 Hz); ^{13}C NMR (pyridine-d_5): δ 159.9, 132.1, 130.2, 128.2, 124.6, 114.6, 104.1, 78.8, 78.7, 75.4, 71.8, 70.1, 62.9, 55.3; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{16}H_{22}O_7$: 327.1444 (M^+ + H); found, 327.1417.

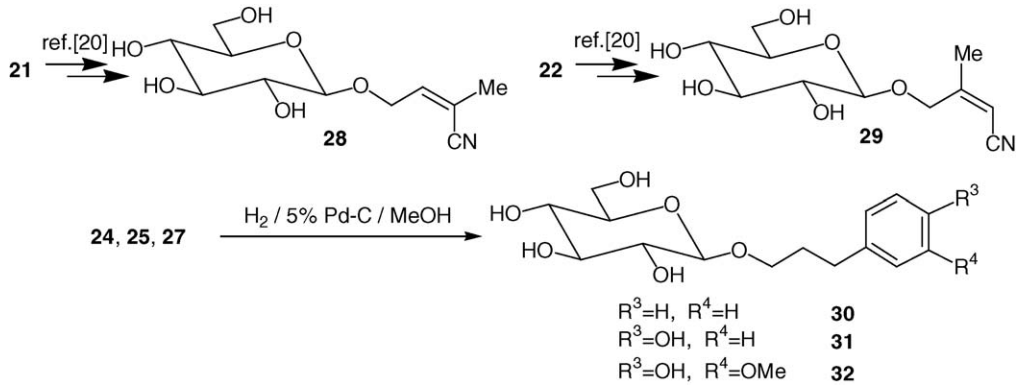
*3.1.2.5. 4-Hydroxy-3-methoxycinnamyl β-D-glucopyranoside (Citrusin D, Coniferin) (**27**).* A mixture of D-glucose **2** (1.1 g, 6.1 mmol), 4-hydroxy-3-methoxycinnamyl alcohol (**9**, 4.4 g, 24.4 mmol), water (3 ml), and the above-mentioned immobilized β-glucosidase (1.1 g, ca. 370 units) in *tert*-butanol (27 ml) was incubated for 7 d at 50 °C. The reaction mixture was filtered off and the filtrate was directly subjected to chromatography on silica gel (150 g) to give 4-hydroxy-3-methoxycinnamyl alcohol (**9**, 4.15 g, 94% recovery) from the $CHCl_3$/MeOH = 30:1 eluent and β-D-glucopyranoside (**27**, 0.167 g, 8% yield) as a pale yellow solid from the $CHCl_3$/MeOH = 10:1 eluent. The NMR (^{1}H and ^{13}C NMR) data of β-D-glucopyranoside (**27**) were identical with those of the reported β-D-glucopyranoside (**27**) [11]. **27**: $[\alpha]_D^{23}$ −36.0 (c = 0.54, MeOH); IR (KBr): 3382, 2862, 1515, 1278, 1032 cm^{-1}, ^{1}H NMR (CD_3OD): δ 3.20-3.38 (4H, m), 3.68 (1H, dd, J = 5.0, 12.1 Hz), 3.86 (3H, s), 3.82–3.90 (1H, m), 4.29 (1H, ddd, J = 2.0, 6.5, 12.6 Hz), 4.36 (1H, d, J = 8.0 Hz), 4.49 (1H, ddd, J = 1.5, 5.6, 12.6 Hz), 6.19 (1H, td, J = 6.0, 16.1 Hz), 6.57 (1H, d, J = 16.1 Hz), 6.73 (1H, d, J = 8.1 Hz), 6.85 (1H, d, J = 2.0, 8.1 Hz), 7.01 (1H, d, J = 2.0 Hz); ^{13}C NMR (CD_3OD): δ 149.1, 147.7, 134.3, 130.4, 123.7, 121.2, 116.2, 110.6, 103.2, 78.1, 78.0, 75.1, 71.7, 71.0, 62.8, 56.4; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{18}H_{26}O_9$: 343.1393 (M^+ + H); found, 343.1365.

*3.2. Conversion of β-D-glucopyranoside (**21**, **22**, **24**, **25** and **27**) into the natural products (**28**–**32**)*

The synthetic β-D-glucopyranoside (**21**) was reported by us to be converted into the cyanoglucoside rhodiocyanoside A (**28**) which was isolated from the underground part of *Rhodiola quadrifida* and found to show antiallergic activity in a passive cutaneous anaphylaxis test in rat [19,20]. The synthetic β-D-glucopyranoside (**22**) was also reported by us to be converted into the cyanoglucoside osmaronin (**29**) which was isolated from a methanolic extract of the leaves of *Osmaronia cerasiformis* [20,21]. Catalytic hydrogenation of the synthetic β-D-glucopyranosides (**24**, **25** and **27**) gave the corresponding hydrogenation products (**30**–**32**), respectively, of which the spectral data were identical with those of natural products (**30**–**32**), respectively [22–24] (Scheme 3).

*3.2.1. 3-Phenylpropyl β-D-glucopyranoside (**30**)*

A mixture of Rosin (**24**; 0.148 g, 0.50 mmol), 5% Pd-C (20.0 mg) in MeOH (5.0 ml) was stirred under H_2 atmosphere at room temperature for 1 h. The reaction mixture was diluted with AcOEt and filtered through Celite. The filtrate was concentrated to give a residue, which was subjected to the flash column chromatography on silica gel [5 g, CH_2Cl_2/MeOH (20:1 to 7:1)] to afford **30** (0.114 g, 76%) as a colorless amorphous. **30**:



Scheme 3. Conversion of **21**, **22**, **24**, **25** and **27** into the natural products **28**–**32**, respectively.

$[\alpha]_D^{29}$ −29.3 (c = 0.75, MeOH); IR (KBr): 3363, 1245, 1030, 700 cm^{-1}, ^1H NMR (CD_3OD): δ 1.88–1.95 (2H, m), 2.71 (2H, t, J = 7.3 Hz), 3.20 (1H, dd, J = 7.6, 8.8 Hz), 3.23–3.28 (1H, m), 3.30 (1H, t, J = 8.3 Hz), 3.36 (1H, dd, J = 8.3, 8.8 Hz), 3.55 (1H, dt, J = 9.6, 6.6 Hz), 3.67 (1H, dd, J = 5.3, 11.9 Hz), 3.86 (1H, dd, J = 2.3, 11.9 Hz), 3.92 (1H, dt, J = 9.6, 6.6 Hz), 4.25 (1H, d, J = 7.6 Hz), 7.12–7.16 (1H, m), 7.18–7.27 (4H, m); ^{13}C NMR (CD_3OD): δ 143.4, 129.5(2C), 129.3(2C), 126.8, 104.5, 78.2, 77.9, 75.2, 71.7, 70.0, 62.8, 33.2, 32.7; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{15}H_{22}O_6$: 299.1549 (M^+ + H); found, 299.1466.

3.2.2. 3-(4-Hydroxy)phenylpropyl β-D-glucopyranoside (31)

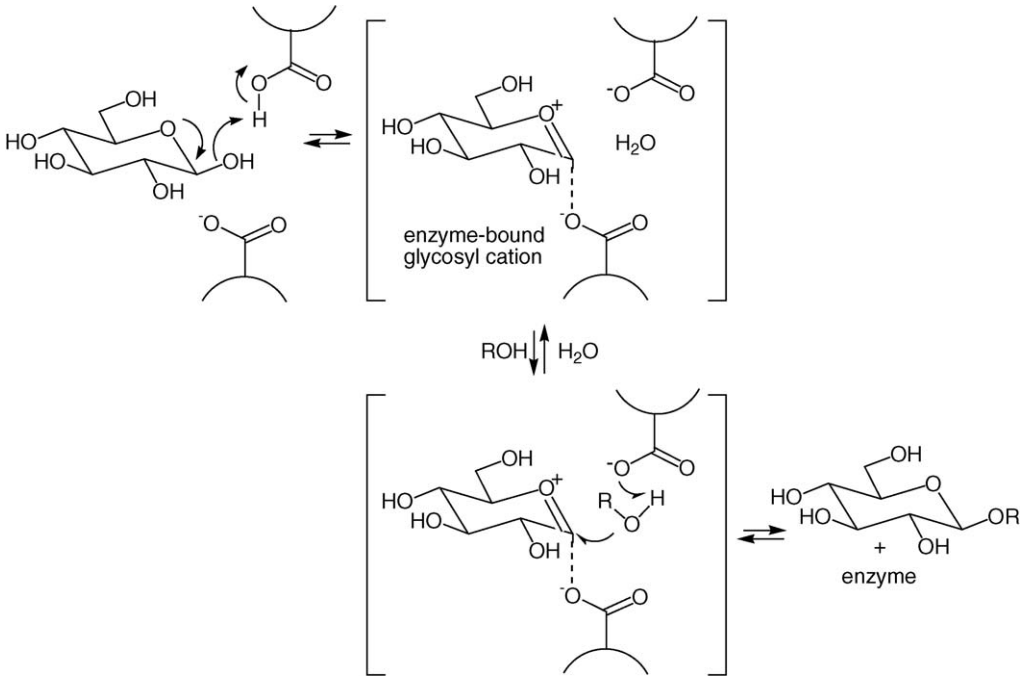
A mixture of Sachaliside I (**25**; 0.156 g, 0.50 mmol), 5% Pd–C (20.0 mg) in MeOH (5.0 ml) was stirred under H_2 atmosphere at room temperature for 1 h. The reaction mixture was diluted with AcOEt and filtered through Celite. The filtrate was concentrated to give a residue, which was subjected to the flash column chromatography on silica gel [5 g, CH_2Cl_2/MeOH (20:1 to 5:1)] to afford **31** (0.105 g, 67%) as a colorless amorphous. **31**: $[\alpha]_D^{30}$ −25.7 (c = 1.8, MeOH); IR (KBr): 3371, 1514, 1236, 1028, 828 cm^{-1}, ^1H NMR (CD_3OD): δ 1.87 (2H, tt, J = 6.6, 7.6 Hz), 2.61 (2H, t, J = 7.6 Hz), 3.19 (1H, dd, J = 7.8, 8.8 Hz), 3.22–3.27 (1H, m), 3.29 (1H, t, J = 8.3 Hz), 3.35 (1H, dd, J = 8.3, 8.8 Hz), 3.53 (1H, dt, J = 9.6, 6.6 Hz), 3.67 (1H, dd, J = 5.6, 11.9 Hz), 3.85 (1H, dd, J = 2.0, 11.9 Hz), 3.90 (1H, dt, J = 9.6, 6.6 Hz), 4.24 (1H, d, J = 7.8 Hz), 6.68 (2H, d, J = 8.6 Hz), 7.02 (2H, d, J = 8.6 Hz); ^{13}C NMR (CD_3OD): δ 156.4, 134.2, 130.4(2C), 116.1 (2C), 104.5, 78.2, 77.9, 75.2, 71.7, 70.1, 62.8, 32.9, 32.3; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{15}H_{22}O_7$: 315.1444 (M^+ + H); found, 315.1424.

3.2.3. 3-(4-Hydroxy-3-methoxyphenyl)propyl β-D-glucopyranoside (32)

A mixture of Citrusin D (**27**; 0.171 g, 0.50 mmol), 5% Pd–C (20.0 mg) in MeOH (5.0 ml) was stirred under H_2 atmosphere at room temperature for 1 h. The reaction mixture was diluted with AcOEt and filtered through Celite. The filtrate was concentrated to give a residue, which was subjected to the flash column chromatography on silica gel [5 g, CH_2Cl_2/MeOH (20:1 to 5:1)] to afford **32** (0.099 g, 56%) as a colorless amorphous. **32**: $[\alpha]_D^{28}$ −18.1 (c = 0.5, MeOH); IR (KBr): 3392, 1496, 1258, 1032, 810 cm^{-1}, ^1H NMR (CD_3OD): δ 1.84–1.92 (2H, m), 2.62 (2H, t, J = 7.6 Hz), 3.19 (1H, dd, J = 7.8, 9.1 Hz), 3.23–3.37 (3H, m), 3.52 (1H, dt J = 9.6, 6.6 Hz), 3.66 (1H, dd, J = 5.6, 12.1 Hz), 3.82 (3H, s), 3.86 (1H, dd, J = 2.2, 12.1 Hz), 3.91 (1H, dt, J = 9.6, 6.6 Hz), 4.24 (1H, d, J = 7.8 Hz), 6.62 (1H, dd, J = 1.8, 8.1 Hz), 6.67 (2H, d, J = 8.1 Hz), 6.77 (1H, d, J = 1.8 Hz); ^{13}C NMR (CD_3OD): δ 150.43, 150.39, 131.6, 122.0, 117.7, 113.2, 104.5, 78.1, 77.9, 75.2, 71.7, 70.3, 62.8, 56.3, 33.0, 32.7; HR-MS (FAB-MS) *m/z*: anal. calcd. for $C_{16}H_{24}O_8$: 345.1549 (M^+ + H); found, 345.1537.

3.3. Discussion

Previously, we reported that the direct β-glucosidation of 3-methyl-2-buten-1-ol (**3**) or 2-methyl-2-propen-1-ol (**4**) using *p*-nitrophenyl-β-D-glucopyranoside (**1**) as glycosyl donor under kinetic condition gave the corresponding β-D-glucopyranosides (**21**) or (**22**) in 25% or 14% yield, respectively [20]. In the present procedure (method I) using high concentration of the alcohol acceptor under equilibrium condition, chemical yield of **21** or **22** was fairly improved to 65 or 51%, respectively. This procedure was applied for the direct glycosidation of 1-octanol, 1-hexanol, (3*Z*)-hexen-1-ol and allyl alcohol to provide the corresponding β-D-glucopyranosides (5% [25], 14% [26], 17% [27] and 68% [28] yields, respectively) and chemical yield was found to be dependent upon the subtle structure of the used alcohol. On the other hand, in case of chemical yields of the direct β-glycosidation using 4-equivalents of cinnamyl alcohol congeners in 90% *tert*-butanol/H_2O solution (method II), chemical yields of β-D-glucopyranosides were not always satisfactory and should be improved. Enzymatic formation of a glycosidic bond is thought to be mechanistically similar to the acid-catalyzed formation of glycosides [29]. The active site of β-glucosidase was constructed with two carboxylic acid parts which play the important role of catalyzing the hydrolysis of glycosidic linkages. One is the carboxylate ion which acts as a general base and the other is carboxylic acid which acts as a general acid. When the substrate is brought close to the active site of the enzyme, the oxocarbenium ion with α-configuration at the anomeric carbon as shown in Scheme 4 was formed. This oxonium ion or the enzyme-bound glycosy cation was stabilized by an ion-pair intermediate or covalent bonding and can



Scheme 4. Plausible mechanism of transglycosylation via glycosyl cation.

be captured by an alcohol to yield a glycoside. Nucleophilic alcohol presumably attacks the anomeric carbon from the β-side to afford exclusively β-D-glucopyranoside.

4. Conclusion

For the purpose of synthesis of naturally occurring β-D-glucopyranoside, direct β-glycosidation for seven kinds of the functionalized primary alcohol in the presence of D-glucose using native or immobilized β-glucosidase (EC 3.2.1.21) from almonds under equilibrium condition was carried out. The utilization of high concentration of the alcohol acceptor, 3-methyl-2-buten-1-ol (**3**) or 2-methyl-2-propen-1-ol (**4**) using the immobilized enzyme gave the corresponding β-D-glucopyranoside (**21**, natural product) or (**22**) in 65 or 51% yield, respectively. On the other hand, the utilization of 4-equivalents of the functionalized alcohols (**5**–**9**) using the immobilized enzyme in 90% *tert*-butanol/H_2O solution afforded the naturally occurring β-D-glucopyranosides (**23**–**27**), respectively, in moderate yield. Among them, five kinds of β-D-glucopyranosides (**21**, **22**, **24**, **25** and **27**) were converted into the cyanoglucosides (rhodiocyanoside A (**28**), osmaronin (**29**)) and other naturally occurring β-D-glucopyranosides (**30**–**32**), respectively.

References

[1] C.-H. Wong, G.M. Whitesides, Enzymes in synthetic organic chemistry Tetrahedron Organic Chemistry Series, vol. 12, Pergamon, Oxford, 1994, p. 252.
[2] K. Faber, Biotransformation in Organic Chemistry: A Text Book, 4th ed., Springer-Verlag, Berlin, 2000, p. 307.
[3] X. Zhang, T. Kamiya, N. Ohtsubo, H. Ishida, M. Kiso, J. Carbohydr. Chem. 18 (1999) 225.
[4] A. Basso, A. Ducret, L. Gardossi, R. Lortie, Tetrahedron Lett. 43 (2002) 2005.
[5] G. Vic, D. Thomas, Tetrahedron Lett. 33 (1992) 4567.
[6] G. Vic, D.H.G. Crout, Tetrahedron: Asymmetry 5 (1994) 2513.
[7] G.G. Zapesochnaya, V.A. Kurkin, Khim. Prir. Soedin. 6 (1982) 723.
[8] G.G. Zapesochnaya, V.A. Kurkin, Chem. Nat. Compd. 18 (1982) 685.
[9] V.A. Kurkin, G.G. Zapesochnaya, Khim. Prir. Soedin. 4 (1991) 481.
[10] M. Mizuno, M. Kato, N. Hosoi, M. Iinuma, T. Tanaka, Heterocycles 31 (1990) 1409.
[11] A. Sawabe, Y. Matsubara, Y. Iizuka, K. Okamoto, Nippon Nogeik. Kaishi 62 (1988) 1067.
[12] S. Fukui, A. Tanaka, Adv. Biochem. Eng./Biotechnol. 29 (1984) 1.
[13] J. Kitajima, T. Ishikawa, Y. Tanaka, Chem. Pharm. Bull. 46 (1998) 1643.
[14] H. Shimomura, Y. Sashida, T. Adchi, Phytochemistry 26 (1987) 2363.
[15] K. Seya, K. Endo, H. Hikino, Phytochemistry 28 (1989) 1495.
[16] H. Nishimura, H. Sasaki, T. Morota, T. Chin, H. Mitsuhashi, Phytochemistry 29 (1990) 3303.
[17] Z.-D. He, S. Ueda, K. Inoue, M. Akaji, M. Fujita, C.-R. Yang, Phytochemistry 35 (1994) 177.
[18] H. Kuwajima, Y. Takai, K. Takaishi, K. Inoue, Chem. Pharm. Bull. 46 (1998) 581.
[19] M. Yoshikawa, H. Shimada, H. Shimada, N. Murakami, J. Yamahara, H. Matsuda, Chem. Pharm. Bull. 44 (1996) 2086.
[20] H. Akita, K. Kurashima, T. Nakamura, K. Kato, Tetrahedron: Asymmetry 10 (1999) 2429.
[21] M. Lechtenberg, A. Nahrstedt, V. Wray, F.R. Fronczek, Phytochemistry 37 (1994) 1039.
[22] K. Kurashima, M. Fujii, Y. Ida, H. Akita, Chem. Pharm. Bull. 52 (2004) 270.
[23] R. Higuchi, M. Aritomo, D.M.X. Donnelly, Phytochemistry 16 (1977) 1007.
[24] G.D. Manners, D.D. Penn, L. Jurd, L.F. James, J. Agric. Food Chem. 30 (1982) 401.
[25] H. Akita, E. Kawahara, K. Kato, Tetrahedron: Asymmetry 15 (2004) 1623.
[26] M. Kishida, M. Nishiuchi, K. Kato, H. Akita, Chem. Pharm. Bull. 52 (2004) 1105.
[27] M. Kishida, M. Fujii, Y. Ida, H. Akita, Heterocycles 65 (2005) 2127.
[28] M. Kishida, H. Akita, Tetrahedron 61 (2005) 10559.
[29] D.G. Drueckhammer, W.J. Hennen, R.L. Pederson, C.F. Barbas, C.M. Gautheron, T. Krach, C.-H. Wong, Synthesis (1991) 499.