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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2095–2097

Use of apple seed meal as a new source of β-glucosidase for enzymatic glucosylation of 4-substituted benzyl alcohols and tyrosol in monophasic aqueous-dioxane medium

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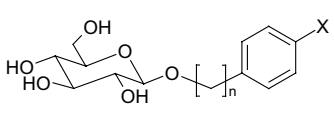
Received 31 December 2003; revised 9 February 2004; accepted 10 February 2004

Abstract—A facile method for enzymatic glycosylation of 4-substituted benzyl alcohols and tyrosol with glucose in a monophasic aqueous-dioxane medium was reported, using a crude meal of apple seed as a new catalyst. The corresponding β-D-glucosides were synthesized in moderate yields (13.1–23.1%), among which the salidroside was obtained in 15.8% yield.

As a part of a program to search for novel glycoside compounds with biological activities, we are interested in synthesizing a series of 4-substituted benzyl glucosides and salidroside (2-(4-hydroxyphenyl)ethyl β-D-glucoside). Glycosidases can catalyze the synthesis of anomerically pure glycosides in one step using a reverse hydrolysis process or a transglycosidation procedure, while chemical synthesis of anomerically pure glucosides is circuitous and expensive. This problem in chemical synthesis has stimulated the development of enzymatic approaches.[1]

Almond β-glucosidase has been widely used to catalyze the synthesis of various alkyl β-glucopyranosides in organic media.[2–6] For the large-scale production of alkyl-β-glucopyranosides, however, it will be necessary to find easily available and relatively cheap catalysts. Many reports described a transglycosylation procedure where an expensive and activated glycosyl donor was used. For example, Akita et al.[2] synthesized a series of β-glucosides using *p*-nitrophenyl glucopyranoside (PNPG) as a glucosyl donor in an aqueous medium and 4-methoxybenzyl β-D-glucopyranoside was formed in 25% yield. The result of the transglucosylation procedure was attractive, but the use of an expensive glycosyl donor increased the cost of the synthesis. There had been relatively few examples of reverse hydrolysis, although it is the simplest and the most cost-effective process. In order to favor the formation of β-glucosides in higher yields, liquid-state alcohols were preferentially used both as substrates and as solvents. For instance, Vic and Crout[3] had synthesized benzyl β-D-glucopyranoside in 40% yield by this method. However, the need to use a very high concentration of the alcohols clearly limits the scope and application of this reaction. Moreover, when an alcohol is a solid, it is necessary to use a solvent in the system to dissolve the substrate. We found that dioxane-aqueous medium was more effective for our reaction than the traditional *tert*-butanol-aqueous or CH_3CN-aqueous system. In this communication, therefore, we describe the synthesis of seven β-glucopyranosides (Scheme 1) through a reverse hydrolysis process starting directly from D-glucose and the corresponding alcohols (in either liquid or solid state) in a monophasic



1 n=1, X=H
2 n=1, X=OCH_3
3 n=1, X=CH_3
4 n=1, X=NO_2
5 n=1, X=F
6 n=1, X=Cl
7 n=2, X=OH

Scheme 1. Structure of 4-substituted benzyl β-D-glucopyranosides and salidroside.

Keywords: β-D-Glucoside; Enzymatic synthesis; Apple seed meal; β-Glucosidase; Salidroside.
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doi:10.1016/j.bmcl.2004.02.042

buffer-dioxane medium. Scigelova et al.[7] screened glycosidases from several enzyme preparations and found a preparation from apples had glucosidase activity. However, to the best of our knowledge, our work first applied the crude meal of apple seeds as a biocatalyst in the synthesis of glycosides **1**, **2**, and **7**. Furthermore, the synthesis of novel glycoside compounds **3**–**6** are reported for the first time.

In the above-mentioned monophasic medium system, the ratio of dioxane to buffer was found to be a sensitive parameter affecting both the reaction rate and the equilibrium yield, and the highest yield was obtained at an optimum ratio of 10:1 (v/v). Under the optimal condition, seven 4-substituted benzyl or phenylethyl β-D-glucopyranosides were successfully synthesized in moderate yields (Table 1) by using the crude meal of apple seeds as a new source of β-glucosidase. It should be noticed that for alcohols **1**, **2**, and **5**, which are in liquid state, the yields were relatively higher (>18%), perhaps due to the better mixing effect between liquid–liquid than that between solid–liquid and for tyrosol **7**, the product of glucosylation, salidroside, was also obtained in 15.8% yield.

The time course of the glucosylation reaction of 4-nitrobenzyl alcohol was monitored by HPLC, as shown in Figure 1. Since the reaction at 20 h had been approaching the equilibrium conversion (ca. 13.3%, as determined from both of the forward and reverse reactions), a sharp decrease in reaction rate was observed around 20 h. As compared with the time course of the reaction by almond β-glucosidase, it is clear that the crude enzyme from the apple seed meal showed a very similar profile. Therefore, the crude enzyme from apple seeds could become a good complement to the source of the almond β-glucosidase. It is expected that the reverse hydrolysis reactions catalyzed by glycoside hydrolases might also be applied for the glycosylation of other solid-state alcohols.

Table 1. Synthesis of β-glucopyranosides by apple seed meal

Entry	*n*	X	Alcohol state	$[Glucose]_0$/M	Yield (%)[a]
1	1	–H	Liquid	0.25	20.4
2	1	$–CH_3O$	Liquid	0.25	23.1
3	1	$–CH_3$	Solid	0.25	16.7
4	1	$–NO_2$	Solid	0.25	13.1
5	1	–F	Liquid	0.25	18.1
6	1	–Cl	Solid	0.25	16.1
7	2	–OH	Solid	0.25	15.8

[a] Isolated yield.

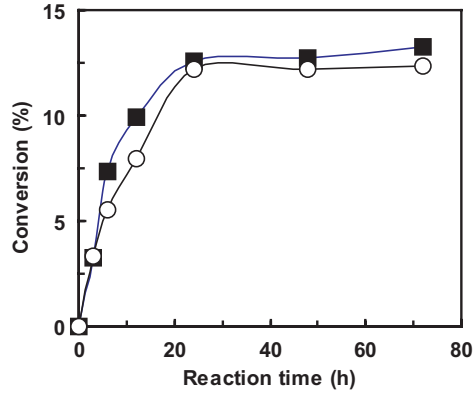

Figure 1. Time-course of 4-nitrobenzyl glucopyranoside formation in buffer-dioxane (1:10, v/v) catalyzed by crude meal of apple seed (■), compared to that by almond β-glucosidase (○). In the reaction system, the crude meal of apple seed added was 50 mg/ml and the almond β-glucosidase added was 5 mg/ml.

All the 4-substituted benzyl alcohols were obtained from Aldrich Chemical Co. Tyrosol was from TCI. All other chemicals were of the highest purity commercially available and used without further purification. Almond β-glucosidase (EC 3.2.1.21) was purchased from Sigma Chemical Co. (3.8 U/mg). Apples were purchased from the local market. NMR spectra were recorded on a Bruker DRX-500 spectrometer or Bruker AM-300 spectrometer. Mass spectra were recorded on a Bruker APEXIII 7.0 TESLA FTMS using ESI mode. IR spectra were recorded on a Digital FTIR instrument. Optical rotations were measured using Perkin–Elmer 241 MC polarimeter.

Benzyl β-D-glucopyranoside (**1**): $[\alpha]_D^{20}$ −55.5 (*c* 0.885, MeOH); ^1H NMR δ (D_2O, ppm) 3.26 (dd, 1H, $J = 8.7$, 8.4 Hz), 3.33 (m, 3H), 3.67 (dd, 1H, $J = 5.6$, 12.4 Hz), 3.87 (dd, 1H, $J = 1.9$, 12.4 Hz), 4.46 (d, 1H, $J = 8.0$ Hz), 4.69 (d, 1H, $J = 11.6$ Hz), 4.88 (d, 1H, $J = 11.6$ Hz), 7.34 (m, 5H); ^{13}C (DMSO): δ 61.2 (C-6), 69.4 (C-4), 70.2 (C-1′), 73.5 (C-2), 76.8 (C-3 or C-5), 76.9 (C-3 or C-5), 102.1 (C-1), 127.2, 127.5, 128.0, 138.0 (C-aryl); FTIR (KBr, cm^{-1}): 3502, 3427, 3062, 2931, 2883, 1497, 1455, 1416, 1382, 1370, 1318, 1280, 1242, 1210, 1157, 1108, 1081, 1051, 1031, 993, 891, 753, 735, 697, 585; *m*/*z* (ESI^+) $C_{13}H_{18}O_6Na$ ($M+Na^+$), 293.0.

4-Methoxybenzyl β-D-glucopyranoside (**2**): $[\alpha]_D^{20}$ −59.4 (*c* 0.88, MeOH); ^1H NMR δ (D_2O, ppm) 3.25 (dd, 1H, $J_1 = J_2 = 8.5$ Hz), 3.33 (m, 3H), 3.69 (dd, 1H, $J = 5.6$, 12.4 Hz), 3.76 (s, 3H), 3.88 (d, 1H, $J = 12.2$ Hz), 4.42 (d, 1H, $J = 8.0$ Hz), 4.60 (d, 1H, $J = 11.3$ Hz), 4.81 (dd, 1H, $J = 11.6$ Hz), 6.92 (d, 4H, $J = 8.6$ Hz), 7.33 (d, 4H, $J = 8.6$ Hz); ^{13}C (DMSO): δ 55.5 (C-Me), 61.7 (C-6), 69.7 (C-4), 70.1 (C-1′), 73.5 (C-2), 77.3 (C-3 or C-5), 77.4 (C-3 or C-5), 102.5 (C-1), 114.0, 129.9, 130.3, 159.2 (C-aryl); FTIR (KBr, cm^{-1}): 3543, 3337, 2955, 2926, 2857, 1613, 1586, 1513, 1367, 1306, 1248, 1165, 1075, 1029, 820, 766, 709; *m*/*z* (ESI^+) $C_{14}H_{20}O_7Na$ ($M+Na^+$), found: 323.1100, calcd for: 3323.1101.

4-Methylbenzyl β-D-glucopyranoside (**3**): $[\alpha]_D^{20}$ −60.3 (*c* 0.98, MeOH); ^1H NMR δ (D_2O, ppm) 2.33 (s, 3H), 3.25 (dd, 1H, $J_1 = J_2 = 8.7$ Hz), 3.34 (m, 3H), 3.68 (dd, 1H, $J = 5.6$, 12.4 Hz), 3.88 (dd, 1H, $J = 1.6$, 12.3 Hz), 4.47 (d, 1H, $J = 8.9$ Hz), 4.68 (d, 1H, $J = 11.5$ Hz), 4.86 (d, 1H, $J = 11.5$ Hz), 7.26 (d, 2H, $J = 7.8$ Hz), 7.33 (d, 2H, $J = 7.9$ Hz); ^{13}C (DMSO): δ 20.9 (C-Me), 61.4 (C-6), 69.5 (C-4), 70.4 (C-1′), 73.7 (C-2), 77.0 (C-3 or C-5), 77.1 (C-3 or C-5), 102.1 (C-1), 127.9, 128.9, 135.1, 136.7 (C-aryl); FTIR (KBr, cm^{-1}): 3356, 2955, 3001, 2925,

2855, 1519, 1463, 1410, 1364, 1311, 1168, 1132, 1112, 1079, 1037, 1015, 897, 799, 748, 650, 613; m/z (ESI^+) $C_{14}H_{20}O_6Na$ ($M+Na^+$), found: 307.1139, calcd for: 307.1152.

4-Nitrobenzyl β-D-glucopyranoside (**4**): $[\alpha]_D^{20}$ −49.5 (c 1.03, MeOH); ^1H NMR δ (D_2O, ppm) 3.32 (m, 4H), 3.69 (dd, 1H, $J = 5.8$, 12.3 Hz), 3.88 (dd, 1H, $J = 2.0$, 12.3 Hz), 4.51 (d, 1H, $J = 7.9$ Hz), 4.82 (d, 1H, $J = 13.1$ Hz), 4.98 (d, 1H, $J = 13.1$ Hz), 7.57 (d, 2H, $J = 8.6$ Hz), 8.14 (d, 2H, $J = 8.7$ Hz); ^{13}C (DMSO): δ 61.2 (C-6), 68.5 (C-4), 70.1 (C-1′), 73.5 (C-2), 76.7 (C-3 or C-5), 77.0 (C-3 or C-5), 102.5 (C-1), 123.3, 128.1, 146.4, 146.7 (C-aryl); FTIR (KBr, cm^{-1}): 3539, 3351, 3250, 3001, 2951, 2925, 2866, 1602, 1514, 1442, 1398, 1348, 1290, 1153, 1080, 1040, 985, 896, 833, 736, 613; m/z (ESI^+) $C_{13}H_{17}O_8NaN$ ($M+Na^+$), found: 338.0835, calcd for: 338.0846.

4-Fluorobenzyl β-D-glucopyranoside (**5**): $[\alpha]_D^{20}$ −55.7 (c 0.98, MeOH); ^1H NMR δ (D_2O, ppm) 3.26 (dd, 1H, $J = 9.0$, 8.1 Hz), 3.34 (m, 3H), 3.69 (dd, 1H, $J = 5.6$, 12.3 Hz), 3.88 (dd, 1H, $J = 2.0$, 12.5 Hz), 4.47 (d, 1H, $J = 8.0$ Hz), 4.67 (d, 1H, $J = 11.6$ Hz), 4.86 (d, 1H, $J = 11.6$ Hz), 7.10 (m, 2H), 7.41 (m, 2H); ^{13}C (DMSO): δ 61.5 (C-6), 69.1 (C-4), 70.5 (C-1′), 73.8 (C-2), 77.1 (C-3 or C-5), 77.3 (C-3 or C-5), 102.4 (C-1), 115.1, 115.3, 134.6, 163.2, 163.5 (C-aryl); FTIR (KBr, cm^{-1}): 3542, 3345, 2859, 2789, 1901, 1604, 1511, 1446, 1368, 1220, 1076, 1031, 896, 829, 776, 617; m/z (ESI^+) $C_{13}H_{17}O_6NaF$ ($M+Na^+$), found: 311.0896, calcd for: 311.0901.

4-Chlorobenzyl β-D-glucopyranoside (**6**): $[\alpha]_D^{20}$ −47.7 (c 0.90, MeOH); ^1H NMR δ (D_2O, ppm) 3.26 (dd, 1H, $J = 8.9$, 8.3 Hz), 3.34 (m, 3H), 3.68 (dd, 1H, $J = 5.7$, 12.3 Hz), 3.88 (dd, 1H, $J = 2.0$, 12.4 Hz), 4.47 (d, 1H, $J = 8.0$ Hz), 4.69 (d, 1H, $J = 11.8$ Hz), 4.87 (d, 1H, $J = 11.8$ Hz), 7.39 (m, 4H); ^{13}C (DMSO): δ 61.4 (C-6), 68.9 (C-4), 70.4 (C-1′), 73.7 (C-2), 76.9 (C-3 or C-5), 77.2 (C-3 or C-5), 102.4 (C-1), 128.4, 129.6, 132.1, 137.4 (C-aryl); FTIR (KBr, cm^{-1}): 3349, 2932, 2874, 1685, 1594, 1493, 1467, 1402, 1362, 1307, 1283, 1208, 1167, 1131, 1111, 1079, 1042, 1014, 897, 832, 810, 799, 763, 637; m/z (ESI^+) $C_{13}H_{17}O_6NaCl$ ($M+Na^+$), found: 327.0605, calcd for: 327.0606.

4-Hydroxyphenylethyl β-D-glucopyranoside (**7**): $[\alpha]_D^{20}$ −30.1 (c 0.50, MeOH); ^1H NMR δ (D_2O, ppm) 2.76 (m, 2H), 3.11 (m, 1H), 3.24 (m, 3H), 3.55 (dd, 1H, $J = 5.8$, 12.4 Hz), 3.74 (dd, 1H, $J = 3.0$, 7.0 Hz), 3.78 (dd, 1H, $J = 1.9$, 12.3 Hz), 3.96 (m, 1H), 4.34 (d, 1H, $J = 8.0$ Hz), 6.75 (d, 2H, $J = 8.5$ Hz), 7.11 (d, 2H, $J = 8.5$).

The fresh apple seeds were peeled off and powdered, washed three times with ethyl acetate, and two times with acetone. The powder was dried in a vacuum desiccator and stored at 4 °C.

General procedure for the enzymatic preparation of glucosides: To 0.5 ml solution containing 1.25 mmol glucose and 0.07 M Na_2HPO_4–KH_2PO_4 (pH 6.0), was added 250 mg crude enzyme. The reaction was started by the addition of benzyl alcohol (15.0 mmol) in 5 ml dioxane and the mixture was shaken at 160 rpm and 50 °C for 72 h. The reaction was quenched by addition of 10 ml methanol. Then the crude enzyme was filtered off and washed with methanol (5 ml × 2). The filtrate was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography on Silica Gel 60 (100–200 mesh) with ethyl acetate/methanol (12:1) as elute, giving β-pyranoglucosides (Table 1) as amorphous solid. Aliquots (50 μl) were removed at time intervals and quenched by addition of 950 μl methanol. The samples were then analyzed by HPLC using YWG-C_{18}-5μ column, eluted with MeOH/H_2O (40:60) and detected at 254 nm.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China (Grant No. 203900506). The authors are grateful to Dr. Pei-Ran Chen for his helpful discussion and technical assistance.

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