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A simple and efficient one-step, regioselective, enzymatic glucosylation of arbutin by α-glucosidase

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Abstract—4-Hydroxyphenyl-β-isomaltoside has been synthesized by α-glucosidase assisted transglycosylation between arbutin as acceptor and sucrose as donor molecules, respectively. Optimum conditions for the transglucosylation reaction were 40 °C for 20 h with 10 mM arbutin and 1.5 M sucrose in a 100 mM sodium citrate/phosphate buffer at pH 5.0. The new glucoside was obtained in a 50% molar yield with respect to arbutin.

In some cases enzymatic synthesis is superior to chemical synthesis, as the enzymatic reactions occur regioselectively and stereoselectively without the need to employ protection and deprotection sequences.[1] In addition, enzymatic reactions proceed under mild conditions at low temperature and neutral pH. Various compounds, such as drugs, vitamins, and phenolic compounds have been glucosylated anomer-selectively with glycosidases from microorganisms.[2]

Although a large number of glycosyl hydrolases are known to perform transglycosylation, the majority of reports have described the use of almond β-glucosidase.[3–5] However, α-glucosidase (maltase) is one of the most abundant glycosyl hydrolases present in Baker's yeast and has been used for the synthesis of menthyl-[6] and *n*-alkyl-glucosides.[7] Various natural glycosides of aromatic compounds have also been prepared using glucosidase. One of them was 4-hydroxyphenyl β-D-glucopyranoside (arbutin), which accumulates in the leaves of plants and is used as a cosmetic ingredient.[8] On the other hand, 4-hydroxyphenyl α-D-glucopyranoside (α-arbutin) has been synthesized enzymatically from hydroquinone and saccharides.[9–11] 4-Hydroxyphenyl β-D-maltoside and 4-hydroxyphenyl β-D-maltotrioside have been synthesized by cyclomaltodextrin glucanotransferase (CGTase)-assisted glucanotransferase transglycosylation between arbutin and starch as acceptor and donor molecules, respectively.[11] In addition, 4-hydroxyphenyl α-D-maltoside and 4-hydroxyphenyl α-D-maltotrioside were synthesized using transglycosylation of CGTase.[12] In our previous studies we examined the stability of maltase[13] and synthesized 4-hydroxyphenyl α-D-isomaltotrioside from hydroquinone and maltose using maltase from baker's yeast.[10]

In this Letter, we report that an α-glucosidase from *Saccharomyces cerevisiae* catalyzes the glucosidation of β-arbutin to produce compound **1**. The reaction between sucrose and arbutin is illustrated in Scheme 1.

The enzymatic reaction was stopped by adding 0.1 M HCl to give pH 3.0 and acetonitrile to give 10% (v/v). The reaction mixture was then centrifuged, and analyzed using an Akta Purifier HPLC (column: Waters Spherisorb 5 μm ODS2 4.6 × 250 mm; mobile phase 10% (v/v) in 1 mM HCl at 1.0 ml/min at 280 nm).

The reaction mixture containing 10 mM arbutin, 1.5 M sucrose and 10U α-glucosidase/ml in 100 ml of 0.1 M sodium citrate/phosphate buffer pH 5.0 was incubated for 20 h at 30 °C. The reaction was stopped and then applied to a column packed with Purolite MN102, a synthetic macroporous polystyrene resin, commercialized by Purolite, Wales, UK. The column was first washed with water and HCl. Retained compounds were

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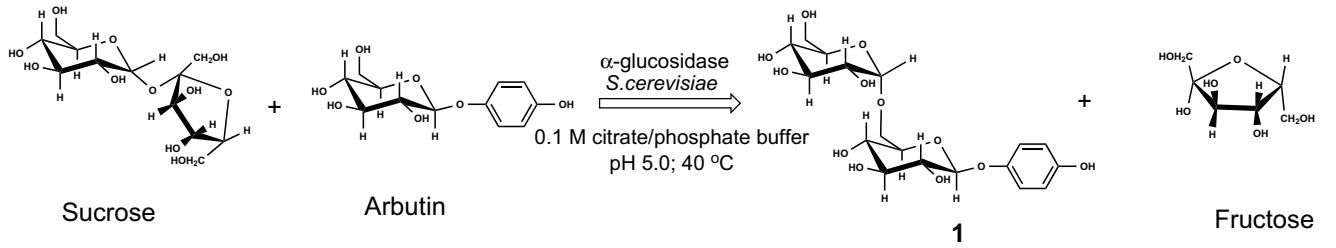

Scheme 1. The transglucosylation reaction catalyzed by α-glucosidase from baker's yeast was optimized with respect to pH, temperature, time and sucrose concentration (Table 1).

eluted with 96% (v/v) ethanol. The eluent was dried by evaporation, then dissolved in distilled water and the residue applied to a Sephadex G-10 column. The fractions were monitored using TLC in ethyl acetate/methanol/water (10:1.7:1.4 by vol) as the solvent. After purification, 400 mg of compound **1** was obtained.

Structural analyses: The product was hydrolyzed to glucose and arbutin with a final molar ratio of 1:1 by α-glucosidase. The TOF LC/MS analysis of the product showed a molecular ion peak in positive mode $[M+Na]^+$at 457.13250 ($C_{18}H_{26}O_{12}$). The specific optical rotation was $[\alpha]_D^{20}$ +0.669. Sixteen signals were observed by ^{13}C NMR analysis. The glycosidic linkages were determined to be of β-configuration and one of α-configuration, based on the values of the coupling constants ($J = 6.6$, $J = 3.6$) of the anomeric protons from the ^1H NMR chemical shift values.[14] From these results, we concluded that the compound was 4-hydroxyphenyl-β-isomaltoside **1**.

Under the conditions described in Table 1, 4-hydroxyphenyl-β-isomaltoside was obtained in a molar yield of 50% with respect to arbutin. This is about twice the yield of the previously reported transglucosylation of *o*-, *m*-, and *p*-hydroxybenzyl alcohols catalyzed by amyloglucosidase.[15] The yield of hydroquinone glucoside obtained was more, 10 times higher, than the previously published results.[9]

By HPLC, only one product was detected in the reaction mixture, and it was estimated that 50% of the arbutin had been glycosylated. We previously reported the synthesis of 4-hydroxyphenyl-α-isomaltoside from hydroquinone and maltose.[10] We purified glycoside **1**, and confirmed its identity as 4-hydroxyphenyl-β-isomaltoside by ^{13}C NMR and ^1H NMR analyses and TOF LC/MS.[16]

In conclusion, a stereospecific synthesis of a new arbutin derivate 4-hydroxyphenyl-β-isomaltoside has been achieved from sucrose and arbutin with yeast α-glucosidase in a one-step reaction. This biocatalyst could be used for this type of reaction with other physiologically active phenolic compounds containing a hydroquinone moiety. Further studies on glucoside **1** with respect to its inhibitory effect on tyrosinase are in progress in our laboratory.

Table 1. Optimal conditions for the transglucosylation reaction of arbutin

Time (h)	pH	*t* (°C)	Sucrose (M)	Arbutin (mM)
20	5.0	40	1.5	10

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16. Analytical data for 4-hydroxyphenyl-β-isomaltoside: TOF LC/MS: $C_{18}H_{26}O_{12}$, the concentration of compound ($c = 0.1$ mg/mL), *ion mass* $(M+Na)^+$ 457.13165, *measured mass* $(M+Na)^+$ 457.13250 error (mDa) 0.84965: $R_f = 0.15$ (ethyl acetate/methanol/water 10:1.7:1.4 v/v), $[\alpha]_D^{20}$ +0.669 ($c = 1.5$ mg/mL, H_2O), ^{13}C NMR (50 MHz, DMSO) 152.6 (C-1), 150.7 (C-4), 118.4 (C-3, C-5), 115.9 (C2, C6), 102.5 (C-1″), 98.6 (C-1′), 78.5 (C-4′), 77.1 (C-3′), 76.5 (C-3″), 75.8 (C-5″), 75.7 (C-2″), 74.5 (C-2′), 74.2 (C-5′), 72.1 (C-4″), 68.3 (C-6′), 63.1 (C-6″), ^1H NMR (200 MHz, DMSO) 6.92 (d, 2H, $J = 9.0$ Hz, H-2, H-6), 6.68 (d, 2H, $J = 9.0$ Hz, H-3, H-5), 4.68 (d, 1H, $J = 3.6$ Hz, H-1′), 4.57 (d, 1H, $J = 6.6$ Hz, H-1″), 3.10–3.70 (m, 12 H, H-2′, H-2″ H-3′, H-3″, H-4′, H-4″, H-5′, H-5″, H-6′A, H-6′B, H-6″, H-6″B).