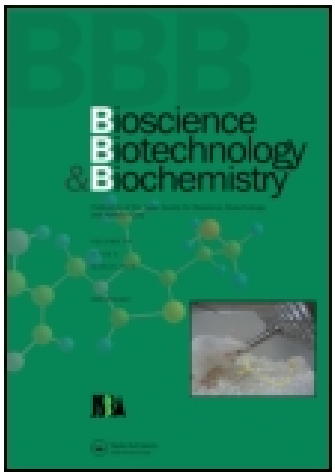

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Effects of α- and β-Arbutin on Activity of Tyrosinases from Mushroom and Mouse Melanoma

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Note

Effects of α- and β-Arbutin on Activity of Tyrosinases from Mushroom and Mouse Melanoma

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The effects of α- and β-arbutin on the activity of tyrosinases from mushroom and mouse melanoma were examined. α-Arbutin was synthesized from hydroquinone and starch using glucoside synthetase (GSase). β-Arbutin inhibited both tyrosinase activities from mushroom and mouse melanoma. α-Arbutin inhibited only the tyrosinase from mouse melanoma, 10 times as strongly as β-arbutin. The IC_{50} of α-arbutin was 0.48 mM and its inhibitory mechanism was speculated to be mixed type inhibition, while that of β-arbutin was noncompetitive.

Substrate analogues for an enzyme often inhibit the corresponding enzyme activity. In the case of tyrosinase, some polyphenols and their derivatives are known to be inhibitors. These tyrosinase inhibitors can be expected to prevent perishable foods from coloring or to suppress melanogenesis in animal cells. For example, hydroquinone-*O*-β-D-glucopyranoside (β-arbutin) which has been found in a plant and used in cosmetics, is known to inhibit tyrosinase activity.[1]

We have synthesized various polyphenol glycosides with glycosyl transfer enzymes to examine their effects on tyrosinase activity. (+)Catechin-α-glucoside, which we synthesized with cyclodextrin glucanotransferase, inhibited the tyrosinase from a mushroom, but didn't inhibit the enzyme from a mouse melanoma.[2] Recently we found GSase, which has potent transfer activity of glucose to polyphenols, from a microorganism.[3] And we studied the effects of polyphenol glucosides, synthesized with GSase, on tyrosinases. In this paper, we describe the enzymatic synthesis of α-arbutin and its effects on the activity of tyrosinases from mushroom and mouse melanoma, and compare them with the effects of β-arbutin.

α-Arbutin was prepared as follows. GSase (1,000 units), which was prepared from *Bacillus subtilis K-531* by the method described previously,[3] was added to 100 ml of 10 mM sodium phosphate buffer solution (pH 6.5) containing 2.5% hydroquinone and 2.5% soluble starch. After incubation at 40°C for 70 hours, 5 mg of glucoamylase (EC 3.2.1.3) from *Aspergillus niger*[4,5] was added to the mixture, and the mixture was incubated at 40°C for 1 h to convert hydroquinone oligoglucoside to hydroquinone glucoside. Then, the mixture was concentrated *in vacuo* to 20 ml. The concentrate was put on a column of Bio-Gel P-2 (2.6 × 90 cm, Bio-Rad Laboratories, Richmond, CA) equilibrated with 5% methanol. A typical chromatogram is shown in Fig. 1. Peak A, eluted before hydroquinone (peak B), contained two compounds. One, which seemed to be α-arbutin glucoside, could be converted to another compound (α-arbutin) by glucoamylase. The fractions containing only α-arbutin was collected and lyophilized. The yield of α-arbutin from 2.5 g of hydroquinone was 150 mg, which was

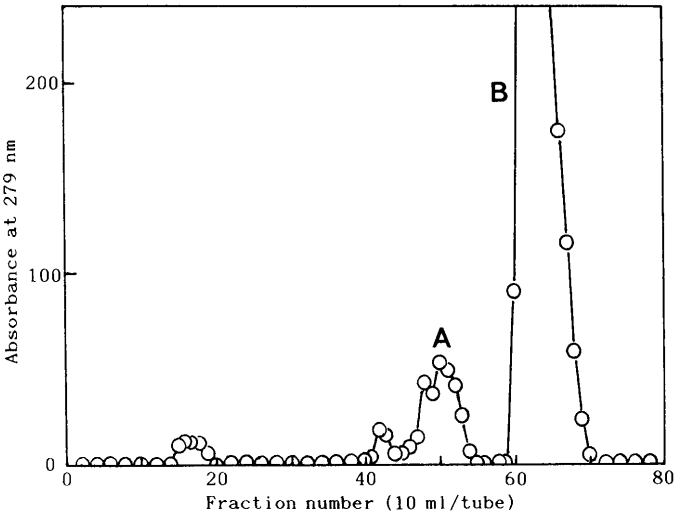

Fig. 1. Chromatogram of the Reaction Products on a Column of Bio-Gel P-2.

The products were eluted at a flow rate of 20 ml per hour and eluates were monitored at A_{279}. Details are described in the text.

Table Comparison of Inhibitory Effect of Hydroquinone Glucosides on the Activity of Tyrosinases

Tyrosinase (EC 1.14.18.1) from *Agricus bisporus* (mushroom) was purchased from Sigma Chemicals Co., St Louis, MO and tyrosinase from mouse melanoma B16-FO (ATCC CRL 6322) was prepared according to a modification of the method of Hashimoto *et al.*[2,6]

The inhibition of tyrosinase activity was measured as in our previous paper.[2] L-DOPA (3-(3,4-dihydroxyphenyl)-L-alanine, 0.83 or 3.3 mM) as substrate and 600 units of tyrosinase was used in each experiment. The increase of the absorbance at 475 nm was measured at 25 or 37°C in sodium phosphate buffer (0.1 M, pH 6.8). The values are concentrations (mM) that inhibit 50% of tyrosinase activity (IC_{50}).

	IC_{50} (mM)	
	Tyrosinase from	
	B16 mouse melanoma[a]	Mushroom[b]
α-Arbutin	0.48	No inhibition[c]
β-Arbutin[d]	4.8	8.4

[a] 3.3 mM L-DOPA was used at 37°C.
[b] 0.83 mM L-DOPA was used at 25°C.
[c] α-Arbutin below 10 mM did not inhibit the activity of tyrosinase.
[d] β-Arbutin was purchased from Nacalai Tesque Co., Kyoto, Japan.

† Corresponding author.

Abbreviations: α-arbutin, hydroquinone-*O*-α-D-glucopyranoside; β-arbutin, hydroquinone-*O*-β-D-glucopyranoside; L-DOPA, 3-(3,4-dihydroxyphenyl)-L-alanine; GSase, glucoside synthetase.

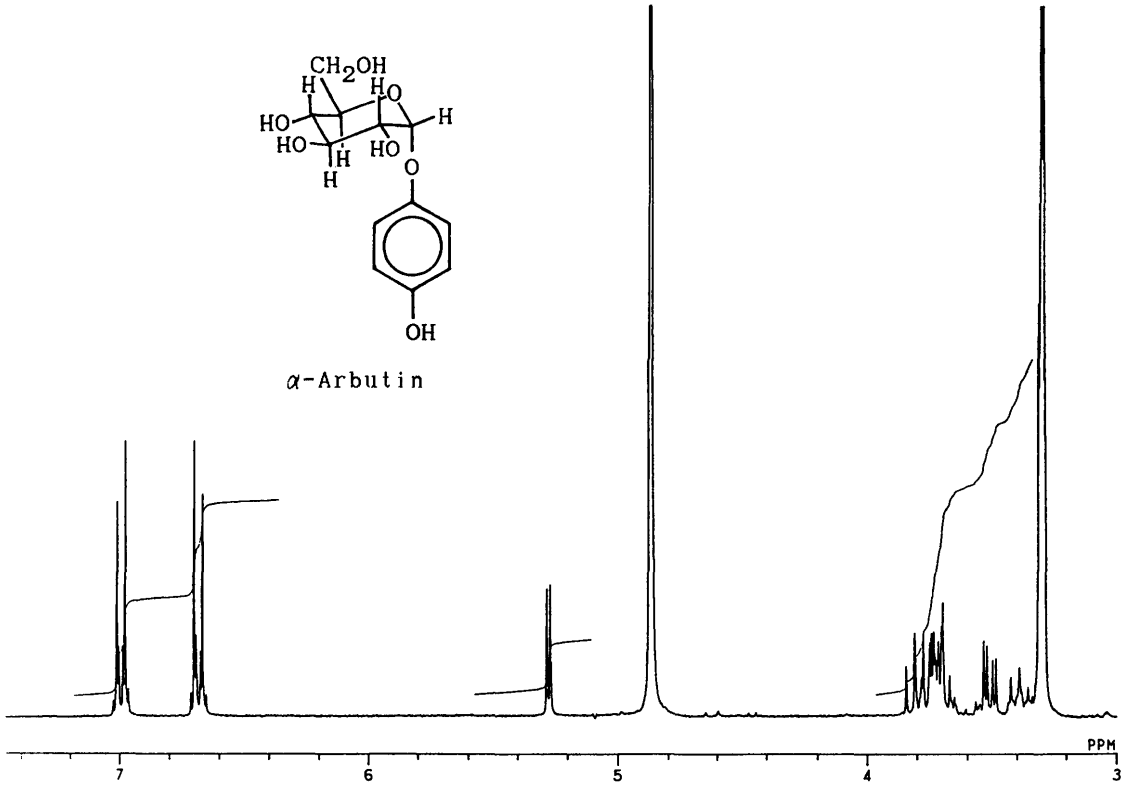

Fig. 2. ^{1}H-NMR Spectrum of the Purified Products.
The spectrum was measured in CD_3OD.

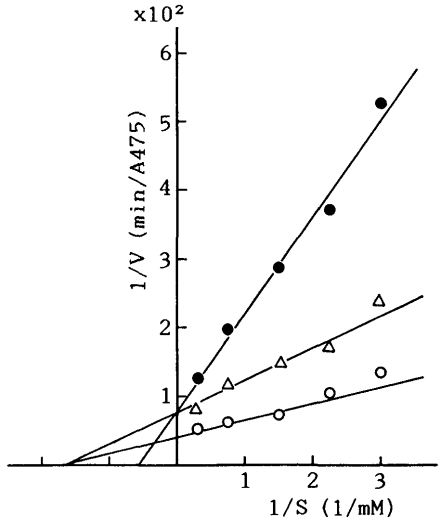

Fig. 3. Lineweaver–Burk Plots of Tyrosinase Activity in the Presence or Absence of Arbutin.
Tyrosinase from mouse melanoma was used. ●, with α-arbutin (0.5 mM); △, with β-arbutin (3 mM); ○, without arbutin.

homogeneous on thin layer chromatography and high performance liquid chromatography.[3)]

The ^{1}H-NMR spectrum of the purified product showed the presence of hydroquinone and glucose in a molar ratio of 1 : 1 as shown in Fig. 2. The α-configuration of the anomeric carbon in glucose could be assigned on the basis of the coupling constant (J=3.8 Hz) of the anomeric proton (δ=5.28) in the ^{1}H-NMR spectrum. From these results, we confirmed that the product was hydroquinone-*O*-α-D-glucopyranoside, *i.e.*, α-arbutin.

α-Arbutin did not inhibit the tyrosinase from mushroom, while its optical isomer, β-arbutin, inhibited the tyrosinase as shown in the Table. On the other hand, α-arbutin inhibited the tyrosinase from mouse melanoma more strongly than the β-arbutin. The IC_{50} of α-arbutin and β-arbutin against the tyrosinase from mouse melanoma were 0.48 mM and 4.8 mM, respectively.

According to Lineweaver–Burk plots of tyrosinase activity, the inhibitory mechanism of α-arbutin and β-arbutin against the tyrosinase from mouse melanoma were speculated to be of the mixed type and the noncompetitive inhibition respectively as shown in Fig. 3. These results suggest that α-arbutin has an affinity for the active site of the mouse melanoma tyrosinase besides the other site.

These results suggested that manipulation of the configuration of the anomeric carbon in a sugar of polyphenol glycoside could change the functions of the glycoside. This investigation suggests that the functions of polyphenol glycosides could be developed with glycosyl transfer enzymes.

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