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Chemical synthesis and tyrosinase inhibitory activity of rhododendrol glycosides

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ABSTRACT

The concise synthesis of rhododendrol glycosides **3**–**8**, which are novel derivatives of (+)-epirhododendrin (**1**) and (−)-rhododendrin (**2**), has been achieved in six steps from benzaldehyde **9**. The key reactions include aldol condensation and trichloroacetimidate glycosylation. From biological studies, it has been determined that synthetic derivatives of **1** and **2** possess potent tyrosinase inhibitory activity. Particularly, the inhibitory activity of cellobioside **8** (IC_{50} = 1.51 μM) is six times higher than that of kojic acid. The *R*-epimers (**4**, **6**, and **8**) possessed more potent activity than the corresponding *S*-epimers (**3**, **5**, and **7**), indicating that tyrosinase inhibitory activity is significantly governed by stereochemistry of rhododendrol glycosides.

Tyrosinase (EC 1.14.18.1), a copper-containing oxidoreductase, catalyzes the enzymatic oxidation of phenol to *o*-quinone.[1–4] The generated *o*-quinone possesses high reactivity and is readily transformed via nonenzymatic and enzymatic processes into colorized biological polymers, including melanin in mammals. Although these polymers may prevent genetic injuries due to excessive exposure to UV irradiation, it results in significant deposition of pigments on the skin. The presence of the oxidation products of L-tyrosine has been linked to the demise of neurons in several neurodegenerative disorders such as Parkinson's disease and Huntington's disease.[5,6] From the agricultural point of view, this browning process by tyrosinase is considered to be deleterious to the color quality of plant derived foods and beverages.[7] Moreover, tyrosinase inhibition leads to preservation of biological activity in foods and medicinal plants,[8,9] because a number of phenolic principles are decomposed by this widely distributed oxidoreductase.[10–12] Thus, adequate control of tyrosinase activity is of considerable importance for preventing cutaneous light aging and neurodegeneration, and preserving food quality.[13]

Acer nikoense so-called 'megusurinoki' (Japanese), is a medicinal plant grown naturally in Japan. This Aceraceae plant has been used as a folk medicine to treat eye diseases for centuries. Nagai and coworkers (1978) isolated (+)-epirhododendrin (**1**), a rhododendrol glycoside from the bark of *A. nikoense* (Fig. 1).[14] The aglycon of **1** has a unique structure because of its *S* stereogenic center, and (−)-rhododendrin (**2**), which is an epimer of **1** and its aglycon have been found frequently in secondary plant metabolites.[15–19] Chemo- and bio-synthetic studies of the glucosides **1** and **2** have been performed by several research groups.[20–23] However, the synthesis of derivatives based on the rhododendrol scaffold to furnish novel physiologically active substances has been rarely investigated to date.

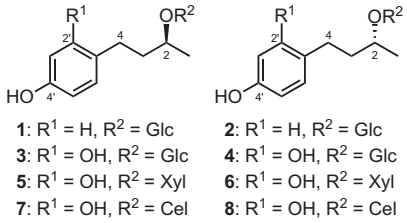

Figure 1. Structure of rhododendrol glycosides **1**–**8**.

The monophenol structure in glucosides **1** and **2** is potentially a good substrate for tyrosinase.[24,25] In contrast, several hydrophobic alkyl resorcinols have been shown to be effective inhibitors of tyrosinase oxidation.[26,27] Accordingly, the structural modification of glucosides **1** and **2** into resorcinol derivatives may provide a route to the development of novel, potent, and hydrophilic tyrosinase inhibitors. Thus, in this Letter, we conducted the concise synthesis of novel rhododendrol glycosides **3**–**8** and evaluated and reported the results of their tyrosinase inhibitory activity.

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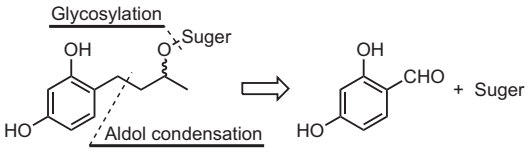


Figure 2. Synthetic plan for rhododendrol glycosides.

The rhododendrol glycosides were synthesized via trichloroacetimidate or Koenigs–Knorr glycosylation from the corresponding sugar donor and aglycon (Fig. 2). Aldol condensation was an appropriate reaction for the preparation of the aglycon from acetone and benzaldehyde **9**.[28] According to the plan, enone **10** was obtained in 94% yield under basic conditions (Scheme 1). Hydrogenation of enone **10** using a palladium ethylenediamine complex on activated carbon [Pd/C(en)] as catalyst and toluene as solvent at 10 °C afforded ketone **11** in 80% yield that was reduced by $NaBH_4$ to afford aglycon **12** in 98% yield.[29] Notably, hydride reduction of enone **10** followed by hydrogenation to yield aglycon **12** failed because the allylic alcohol obtained in the first step was quite labile and was readily converted to the corresponding conjugated diene via dehydration. Moreover, the reduction of **10** with $NaBH_4$ in the presence of $CoCl_2$ did directly provide the desired alcohol because the reaction was sluggish and did not go to completion.[30]

Trichloroacetimidate glycosylation of aglycon **12** using imidate **13** as the glucosyl donor and BF_3 etherate as the Lewis acid did not, unfortunately, afford the desired glycoside **14**. The acetylated compound of aglycon **12** was obtained as the major product using silica gel column chromatography;[31] however, the use of trimethylsilyl trifluoromethanesulfonate instead of BF_3 etherate significantly improved the yield of glycoside **14** to 50%.[32] On the other hand, Koenigs–Knorr glycosylation of aglycon **12** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and Ag_2CO_3 provided glycoside **14** in poor yield (12%).[33]

Hydrogenolysis of glycoside **14** was achieved using a catalytic amount of $Pd(OH)_2$ on activated carbon in a hydrogen atmosphere to give the desired diphenol in 82% yield and it was converted to rhododendrol glucosides **3** and **4** by transesterification with sodium methoxide in 89% yield. Consequently, glucosides **3** and **4** were synthesized as an epimeric mixture from **9** in six steps with 27% overall yield.

Next, the high performance liquid chromatography (HPLC) conditions for optimal separation of glucosides **3** and **4** were examined. After several trials, glucosides **3** and **4** were isolated using preparative reverse-phase (RP)-HPLC with an isocratic elution.[34] The stereochemistry at the oxymethine of the aglycon in glucosides **3** and **4** was determined as *S* and *R*,[35] respectively, by comparing the ^1H and ^{13}C nuclear magnetic resonance (NMR) data for the new glucosides with previously reported NMR data for of glucosides **1** and **2**.[18]

The tyrosinase inhibitory activity of glucosides **3** and **4** was tested along with that of kojic acid, a commercially available inhibitor, and glucosides **1** and **2**. Because of the unavailability of enantiomerically pure **1** and **2**, an epimeric mixture of these two glucosides was prepared from raspberry ketone as the starting material. The $NaBH_4$ reduction of the benzyl ether of raspberry ketone followed by trichloroacetimidate glycosylation using BF_3 etherate gave the fully protected glucosides of **1** and **2** in 43% yield in three steps.[33] Glucosides **1** and **2** were then obtained in high yield (84%, two steps) after removal of all of the protective groups using the above-described process.[36] Unfortunately, glucosides **1** and **2** could not be separated using several purification methods, such as HPLC and recrystallization.

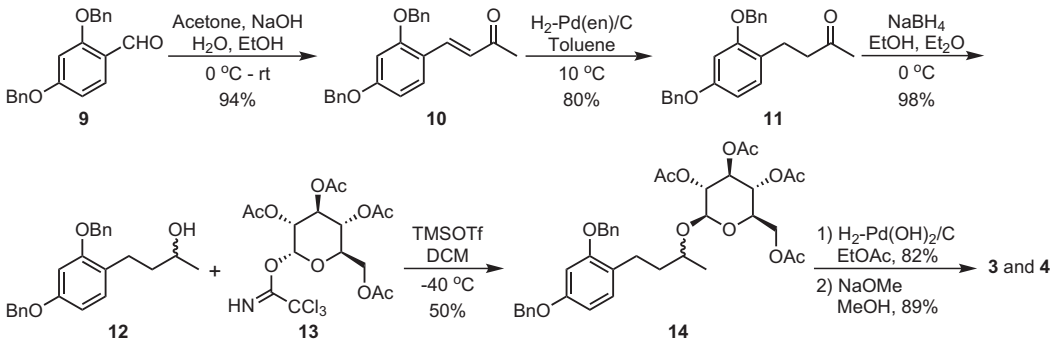
IC_{50} of the mixture of glucosides **1** and **2** could not be estimated against tyrosinase oxidation even when the concentration was increased to 100 μM (Table 1).[37,38] The IC_{50} values for glucosides **3** and **4** were found to be 4.72 and 2.30 μM, respectively, and both were more potent inhibitors than kojic acid (IC_{50} = 9.15 μM), although 4-hexylresorcinol,[27] a hydrophobic inhibitor, exhibited strong activity (IC_{50} = 0.56 μM). Moreover, the IC_{50} of **3** was twice as high as that of **4**, indicating that the stereochemistry of the rhododendrol-type inhibitor played a significant role in enabling the binding of the glucosides to tyrosinase. This result prompted us to synthesize additional rhododendrol glycosides **5**–**8** for a closer investigation of the relationship between the structure of these compounds and their tyrosinase inhibitory activity (Scheme 2).

Thus, trichloroacetimidate glycosylation of aglycon **12** with imidate **15** was carried out at low temperature (−40 °C) to afford xyloside **16** in 48% yield. Removal of all of the protective groups of xyloside **16** via hydrogenolysis and transesterification proceeded smoothly to give rhododendrol xylosides **5** and **6** as an epimeric

Table 1
Tyrosinase inhibitory activities of rhododendrol glucosides **1**–**8**, kojic acid, and 4-hexylresorcinol

Compounds tested	IC_{50}[a] (μM)
1 and **2**	>100
3	4.72 ± 0.58
4	2.30 ± 0.15
5	4.56 ± 0.48
6	1.72 ± 0.17
7	3.83 ± 0.45
8	1.51 ± 0.10
Kojic acid	9.15 ± 0.71
4-Hexylresorcinol	0.56 ± 0.02

[a] The IC_{50} values represent means ± SE of three different experiments.



Scheme 1. Synthesis of rhododendrol glucosides **3** and **4**.