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Chemical synthesis, redox transformation, and identification of sonnerphenolic C, an antioxidant in *Acer nikoense*

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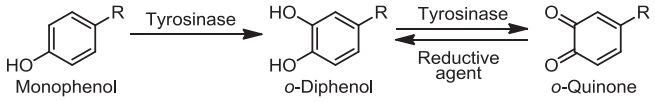
ABSTRACT

Sonnerphenolic C (**3**), which was predicted in a redox product of epirhododendrin (**1**) isolated from *Acer nikoense*, was synthesized for the first time via the epimeric separation of benzylidene acetal intermediates as a key step. From a similar synthetic route, **1** was obtained concisely. As a result of their antioxidative evaluation, only **3** revealed potent activity. The redox transformation of **1** into **3** was achieved in the presence of tyrosinase and vitamin C. Moreover, **3** was identified in the decoction of *A. nikoense* by HPLC analysis with the effective use of synthesized **3**. Thus, a novel naturally occurring antioxidant **3** was developed through the sequential flow including redox prediction, chemical synthesis, evaluation of the activity, and identification as the natural product.

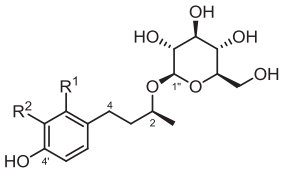
Tyrosinase (polyphenol oxidase, EC 1.14.18.1), a copper-containing oxidoreductase, is widely distributed in nature. Its active site recognizes various phenolic compounds as substrates, and consequently, corresponding *o*-quinones are generated by this enzymatic oxidation (Scheme 1).[1,2] The *o*-quinones are generally labile and can be easily transformed into biopolymers including melanin. Melanin formation is related to skin aging, skin cancer, and Parkinson disease.[3–5] Accordingly, various melanogenesis-controlling agents have been developed from extensive research on organic synthesis and/or natural product chemistry.[6–10]



Scheme 1. Redox transformation of phenolic compounds in the presence of tyrosinase and reductive agent.

In the presence of reductive agents such as vitamin C, the generated *o*-quinones are readily reduced to stable *o*-diphenols.[11] The redox transformation of polyphenols occurs frequently in plant materials because they are rich sources of tyrosinase, phenolic compounds, and reductive agents.[12] Several *o*-diphenols have been identified in nature, with their biological activities clearly differing from those of the corresponding monophenols.[13,14] For instance, the antioxidant activity of *o*-diphenols is preferred to that of their monophenols.[15,16] Thus, this redox transformation can be meaningful for producing unique *o*-diphenols as natural antioxidants.

Acer nikoense (Aceraceae), called "Megusurinoki" in Japanese, is a folk medicinal plant used for curing eye disease.[17] Epirhododendrin (**1**) is a characteristic monophenol in *A. nikoense* (Fig. 1),[18] while several phenolic compounds have been isolated from this plant.[19–23] Recently, a novel and potent tyrosinase inhibitor, rhododendrol glycoside **2**, was developed by the structural modification of **1**.[9,24] This suggests that **1** can easily move closer to the active site of tyrosinase. When **1** acts as a substrate of tyrosinase, an *o*-diphenol sonnerphenolic C (**3**) is possibly generated with the aid of appropriate reductive agents.



1: $R^1 = R^2 = H$
2: $R^1 = OH$, $R^2 = H$
3: $R^1 = H$, $R^2 = OH$

Fig. 1. Structure of **1**–**3**.

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Recently, **3** was isolated from *Sonneratia ovata* (Sonneratiaceae) which showed selective and moderate cell-growth inhibitory effect against MCF-7, a hormone-responsive breast cancer cell.[25] However, the existence of **3** in *A. nikoense* and its antioxidant potency are still unclear. Accordingly, we studied concise syntheses of **1** and **3** via glycosylation and epimeric separation as key steps, and evaluated their antioxidant activity. In addition, redox conversion of **1** to **3** and identification of **3** in the extract of *A. nikoense* were investigated by the effective use of synthetic compounds.

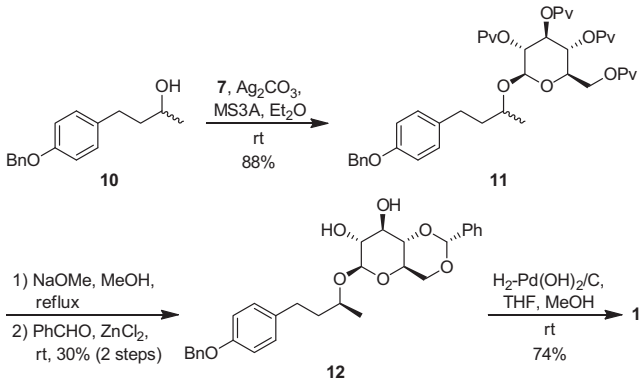
Through aldol condensation between aldehyde **4**[26] and acetone under the basic aqueous condition, enone **5** was obtained in 91% yield (Scheme 2).[27] In the presence of sodium borohydride and cobalt chloride hydrate, reduction of **5** smoothly proceeded to furnish alcohol **6** in 84% yield,[28] although this reaction was sluggish in the synthesis of **2**.[9,24] A comparison of 2,4-dibenzyloxy derivatives indicates that the effects of steric hindrance and/or electronic donation from the 3,4-dibenzyloxy group are insignificantly influenced on its enone part. Alternatively, selective hydrogenation using a palladium ethylenediamine complex on activated carbon, followed by hydride reduction, has been applied in the transformation of **5** into **6** in 90% yield.[9,24] However, the reduction with sodium borohydride and cobalt chloride hydrate was selected in order to reduce synthesis steps and to avoid the dissolution problem of **5** in toluene which is an appropriate solvent for selective hydrogenation.[24]

Glycosylation of **6** was performed under the Koenigs-Knorr condition using pivalate **7** as the glucose donor.[29] Accordingly, β-glucoside **8** was furnished in high yield (93%). In this step, diethyl ether, rather than dichloromethane, acted as an appropriate solvent, whereas the use of ethereal solvents was unsuitable for increasing the production rate of α-glucoside.[30] Schmidt glycosylation[31] and Koenigs-Knorr glycosylation using acetylated sugar failed to obtain the corresponding β-glucosides and complex mixtures were detected by TLC analysis.

Removal of all pivalyl groups in **8** with sodium methoxide, followed by acetalization with benzaldehyde in the presence of Lewis acid, afforded diol **9** in 38% yield over two steps. Epimeric mixtures of **8** as well as the corresponding epimeric intermediates without pivalyl groups could not be separated by various methods including crystallization and flash chromatography. In contrast, **9** and its epimer, which was obtained in 33% over two steps, were easily isolated by silica gel column chromatography.[32] The stereochemistry of the aglycone part of **9** was determined as *S* by comparison with the previously reported ^{1}H data of similar phenolic glucosides.[33]

Finally, catechol **3** was furnished from **9** in excellent yield (99%) by hydrogenolysis using Pearlman's catalyst.[34] The ^{1}H and ^{13}C NMR data of synthesized **3** were fully consistent with those of natural **3**.[25] Consequently, **3** was synthesized in 27% yield from **4** over six steps for the first time. However, the sign of the specific rotation of synthesized **3** ($[\alpha]_D^{27}$ −17.5, *c* 1.0, MeOH) was different from that of natural **3** ($[\alpha]_D^{25}$ +33.4, *c* 1.8, MeOH).[25] According to previous reports,[24,29,32] all congeners such as **1** and **2**, and their epimers, possessed the negative sign, suggesting that unanticipated errors occur in the measurement step of the specific rotation value of natural **3**.

Although asymmetric synthesis of **1** has been accomplished via the enzymatic procedure,[29] the application of the synthetic scheme of **3** led to a concise preparation of **1** (Scheme 3). Racemic alcohol **10**[35] was glycosylated in 88% yield by Koenigs-Knorr method using **7** as the glucose donor. The β-glucoside **11** obtained was converted to diol **12** ($[\alpha]_D^{27}$ −20.2, *c* 0.23, MeOH) in 30% yield over consecutive steps including transesterification and benzylidene acetalizaion. By using silica gel column chromatography, **12** was isolated as a single isomer, which was confirmed by comparison with the NMR data of analogs of **12**.[24,31] Hydrogenolysis of **12** afforded **1** in 74% yield. ^{1}H and ^{13}C NMR data of **1** were fully consistent with those reported previously.[36] Therefore, **1** was synthesized from **10** in 20% over four steps.



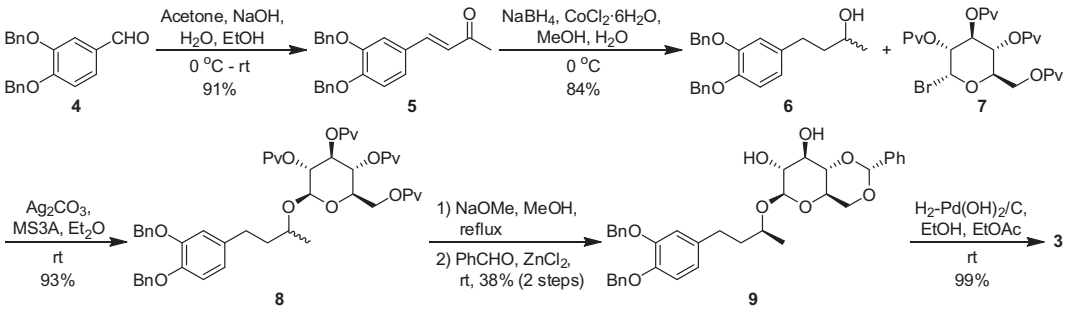
Scheme 3. Synthesis of **1**.

Antioxidant activities of **1** and **3** were evaluated using 1,2-diphenyl-2-picrylhydrazyl (DPPH) (Table 1).[37] Monophenol **1** was inactive to free-radical scavenging, while 1 mol of diphenol **3** eliminated 2.23 mol of DPPH.[38–40] This efficacy was comparable to those of vitamin C and vitamin E. As observed above, the

Table 1
Antioxidant activity of **1**, **3**, vitamin C, and vitamin E.

Compounds tested	DPPH consumption[a]
1	0
3	2.23 ± 0.08
Vitamin C	2.47 ± 0.19
Vitamin E	1.86 ± 0.31

[a] The values indicate that one molecule of the tested compound scavenges how many molecules of DPPH, and represent means ± SE of three different experiments.



Scheme 2. Synthesis of **3**.