The First Total Synthesis of 2,3,6-Tribromo-4,5-dihydroxybenzyl Methyl Ether (TDB) and Its Antioxidant Activity

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Several bromophenols were isolated from Symphyocladia latissima (Harvey) Yamada, which is a member of the family Rhodomelaceae. The antioxidant activity, of the methanolic extract of the S. latissima, on peroxynitrite (ONOO⁻) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was reported by us. We also isolated 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) from this methanolic extract and identified the structure depending on spectroscopic evidence. The reaction of nitric oxide and superoxide generates peroxynitrite, which is a cytotoxicant. Peroxynitrite can oxidize sulfhydryls, lipids, amino acids, and nucleotides. It was demonstrated that excessive formation of peroxynitrite may cause Alzheimers disease, rheumatoid arthritis, cancer, and atherosclerosis.

2,3,6-Tribromo-4,5-dihydroxybenzyl methyl ether

In this report, we reveal the first total synthesis of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether and its scavenging activity of peroxynitrite and DPPH radicals.

The synthetic route of the 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (6) began with the protection of 3,4-dihydroxybenzaldehyde (1) with aceton and phosphorus pentoxide in toluene to generate 2,2-dimethyl-1,3-benzodioxole-5-carboxaldehyde (2) in 85% yield. Aldehyde 2 was reduced with diisobutylaluminum hydride in CH₂Cl₂ at -78 °C to provide alcohol 3 in 85% yield. (2,2-Dimethyl-1,3-benzodioxol-5-yl)methanol (3) was brominated using bromine in concentrated HCl to afford tetraphenyl compound 4 in 7% yield. In this reaction, the unwanted compound dibrominated on the benzene ring was isolated as the major product with 40-50% yield. The resulting 4,5,7-tri­bromo-6-(bromomethyl)-2,2-dimethyl-1,3-benzodioxole (4) was treated with calcium carbonate in aqueous dioxane to provide alcohol 5 in 84% yield. Finally, (4,6,7-t­ribromo-2,2-dimethyl-1,3-benzodioxol-5-yl) methanol (5) was treated with concentrated HCl and MeOH for 8h at reflux to generate the desired final natural product 2,3,6-tribromo-4,5-dihyroxybenzyl methyl ether (TDB) (6) in 22% yield. In the last step, we tried to improve the yield by using numerous conditions, which led to the debromination of the aromatic bromo groups. The spectroscopic data of the synthesized natural product 6 was identical with that of the naturally occurring 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether.

The synthesized natural product TDB (6) was assayed for its ability to scavenge peroxynitrite (ONOO⁻) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Table 1). As shown in Table 1, the DPPH radical scavenging activity of the synthesized natural product TDB (6) (IC₅₀ = 7.8 μM) appears to be slightly higher than that of the naturally occurring TDB (IC₅₀ = 10.5 μM). This is possibly caused by the impurity in the sample of the naturally occurring TDB. This result also confirms that TDB has higher antioxidant activity as compared with L-ascorbic acid (IC₅₀ = 28.44 μM). Moreover, the synthesized natural product TDB (6) shows strong scavenging...
Table 1. Peroxynitrite and DPPH Radical Scavenging Effect of Synthetic TDB 6

<table>
<thead>
<tr>
<th></th>
<th>DPPH</th>
<th>ONOO⁻</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (µM)</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>Synthetic TDB 6</td>
<td>7.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Natural TDB</td>
<td>10.5</td>
<td>0.013</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>28.4</td>
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</tr>
<tr>
<td>Pentamerilamine</td>
<td>3.36</td>
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</tbody>
</table>

*All the values are stated as the mean of at least three determinations.

Table 1 shows the peroxynitrite (ONOO⁻) (IC₅₀=0.012 µM) and DPPH radical scavenging activity.

In conclusion, we firstly synthesized 2,3,6-tribromo-1,5-di-hydroxy-benzyl methyl ether (TDB) and confirmed that TDB has strong peroxynitrite and DPPH radical scavenging activity.

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References and Notes

10. Selected data for synthetic TDB: white powder, mp 124-125 °C, F 279 (CH₂Cl₂); [α]D = 8.2 (c = 1, MeOH-CH₂Cl₂); 1H-NMR (500 MHz, CDCl₃) δ 3.40 (s, 3H), 4.81 (s, 2H); 13C-NMR (125 MHz, CDCl₃) δ 58.9, 76.9, 114.7, 115.2, 119.7, 130.1, 145.1, 146.9; HRMS HRAB MS \( m/z \) 387.7945 (calculated for C₁₀H₁₃Br₂O₂S, 387.7945).
11. DPPH Radical Scavenging Effect: The DPPH radical scavenging effect was evaluated according to the method of Blois. Methanol solution (4 mL) of various sample concentration (1.5-45 µM) was added to 1 mL DPPH methanol solution (1.5 M). After mixing gently and leaving for 30 min at room temperature, the optical density was measured at 520 nm using a spectrophotometer. The antioxidant activity of each sample was expressed in terms of IC₅₀ (µM) required to inhibit DPPH radical formation by 50% and calculated from the log-dose inhibition curve.
12. Peroxynitrite Scavenging Effect: Peroxynitrite scavenging effect was measured by monitoring the DHR 123 according to a modification of the method of Kooy et al. The stock solution of DHR 123 (5 mM) in dimethylformamide was purified with nitrogen and stored at -80 °C. The working solution with DHR 123 (1 mL, final concentration, 5 µM) diluted from the stock solution was placed on ice in the dark immediately prior to the study. The buffer of 90 mM sodium chloride, 30 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 µM (f.c.) diethylamine N,N-diethyl-methane tetramine acid (DTPA) was purified with nitrogen and placed on ice before use. Peroxynitrite scavenging by the oxidation of DHR 123 was measured with a microplate fluorescence spectrophotometer FL 500 (Bio-Tek Instruments) with excitation and emission wavelengths of 485 and 580 nm, respectively, at room temperature. The background and final fluorescent intensities measured 1 min after treatment with or without SIN-1 (i.e., 10 µM) or authentic peroxynitrite (i.e., 10 µM) in 0.3 N sodium hydroxide. Oxidation of DHR 123 by decomposition of SIN-1 gradually increased, whereas authentic peroxynitrite rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time.