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

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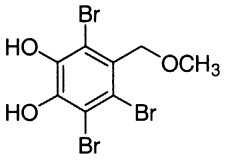
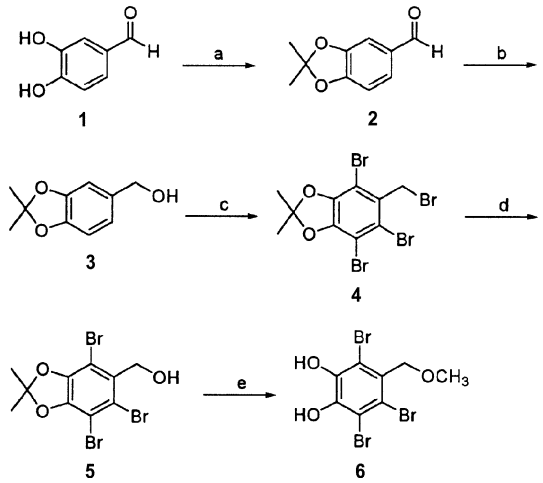
Several bromophenols were isolated from *Symphyocladia latiuscula* (Harvey) Yamada, which is a member of the family Rhodomelaceae.[1-4] The antioxidant activity, of the methanolic extract of the *S. latiuscula*, on peroxynitrite ($ONOO^-$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was reported by us.[5,6] 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.[7] The reaction of nitric oxide and superoxide generates peroxynitrite, which is a cytotoxicant. Peroxynitrite can oxidize sulfhyryls, lipids, amino acids, and nucleotides.[8] It was demonstrated that excessive formation of peroxynitrite may cause Alzheimers disease, rheumatoid arthritis, cancer, and atherosclerosis.[9]



2,3,6-tribromo-4,5-dihydroxybenzyl methy ether

In this report, we reveal the first total syntheses 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 acetone and phosphorus pentoxide in toluene to generate 2,2-dimethyl-1,3-benzodioxole-5-carbaldehyde (**2**) in 85% yield. Aldehyde **2** was reduced with diisobutylaluminum hydride in CH_2Cl_2 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 tetrabromo 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



Scheme 1. *Reaction conditions*; a) Acetone, P_2O_5, toluene; b) DIBAL-hexane, CH_2Cl_2, -78 °C; c) Br_2, *conc*. HCl, H_2O; d) $CaCO_3$, H_2O, dioxane; e) *conc.* HCl, MeOH.

with concentrated HCl and MeOH for 8h at reflux to generate the desired final natural product 2,3,6-tribromo-4,5-dihydroxybenzyl 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[10] of the synthesized natural product **6** was identical with that of the naturally occurring 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether.[7]

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).[11,12] As shown in Table 1, the DPPH radical scavenging activity of synthesized natural product TDB (**6**) (IC_{50}=7.8 μM) appears to be slightly higher than that of the naturally occurring TDB (IC_{50}=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 (IC_{50}=28.44 μM). Moreover, the synthesized natural product TDB (**6**) shows strong scaveng-

Table 1. Peroxynitrite and DPPH Radical Scavenging Effect of Synthetic TDB 6[a]

	DPPH	$ONOO^-$
	IC_{50} (μM)	IC_{50} (μM)
Synthetic TDB **6**	7.8	0.012
Natural TDB	10.5	0.013
L-Ascorbic acid	28.4	
Penicillamine		3.36

[a]All the values are stated as the mean of at least three determinations.

ing activity of peroxynitrite ($ONOO^-$) (IC_{50}=0.012 μM) as shown in Table 1.

In conclusion, we firstly synthesized 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) and confirmed that TDB has strong peroxynitrite and DPPH radical scavenging activity.

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10. Selected data for synthetic TDB: white powder, mp 124-125 °C, *Rf* 0.38 (SiO_2, 2% MeOH-CH_2Cl_2); ^{1}H-NMR (500 MHz, CD_3OD) δ 3.40 (s, 3H), 4.81 (s, 2H), ^{13}C-NMR (125 MHz, CD_3OD), δ 58.9, 76.9, 114.7, 115.2, 119.7, 130.1, 145.1, 146.9, HRFAB MS *m/z* 387.7904 (calculated for $C_8H_7Br_3O_3$ 387.7945).
11. **DPPH Radical Scavenging Effect**: The DPPH radical scavenging effect was evaluated according to the method of Blois.[13] 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 IC_{50} (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*.[14] The stock solution of DHR 123 (5 mM) in dimethylformamide was purged with nitrogen and stored at -80 °C. The working solution with DHR 123 (f.c. (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, 50 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 μM (f.c.) diethylenetriamine pentaacetic acid (DTPA) was purged 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 530 nm, respectively, at room temperature. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (f.c. 10 μM) or authentic peroxynitrite (f.c. 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.
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