Note

Synthesis and Antioxidant Activity of 4-[2-(3,5-Dimethoxyphenyl)ethenyl]-1,2-benzenediol, a Metabolite of Sphaerophyta salsula*

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4-[2-(3,5-Dimethoxyphenyl)ethenyl]-1,2-benzenediol (7), a stilbene isolated from Sphaerophyta salsula, was synthesized from 3,4-dihydroxybenzaldehyde (1) in five steps in an overall yield of 33%. The spectral data for synthetic 7 are in good agreement with those of the natural product. Hydroxystilbene 7 showed potent antioxidative activity.

Key words: 4-[2-(3,5-dimethoxyphenyl)ethenyl]-1,2-benzenediol; Sphaerophyta salsula; synthesis; antioxidative activity

Free radicals play a major role in the progression of a wide range of pathological disturbances. Free radicals have been found by the food industry to be responsible for the deterioration of foods during processing and storage.2) In view of this, considerable attention has been given to the addition of antioxidants to food and to the supplementation of antioxidants to biological systems to scavenge free radicals. A large number of natural products have been found to be antioxidants, including vitamin A, vitamin C, flavonoids, phenolic acids, polyphenolic compounds and carotenoids.3) Resveratrol (3,4,5-trihydroxystilbene), a natural product found in grapes, exhibited antioxidative activity.1) The results are being reported in this note.

To resveratrol, we determined the antioxidative activity of 7 by the nitro blue tetrazolium (NBT) method. 13,14) Compound 7 showed potent superoxide-scavenging activity (IC50: 11 μM) in comparison with other known antioxidants: resveratrol (IC50: 360 μM), vitamin E (IC50: 728 μM), vitamin C (IC50: 852 μM) and BHA (butylated hydroxyanisole; IC50: 967 μM). The superior antioxidative activity of new stilbene 7 lends further support to the fact that the catechol system enhances the antioxidative activity.

Experimental

All solvents were dried and distilled before use, and all reagents were procured from commercial sources and used without further purification. Melting point (mp) data were recorded by a Mel Temp melting point apparatus in open capillaries and are uncorrected. UV spectra were recorded by a Shimadzu UV-190 spectrophotometer, IR spectra by a Perkin-Elmer BX1 FTIR spectrophotometer, mass spectra by an Agilent 1100 LCMS instrument, and elemental analysis by a Heraeus CHN-O-Rapid

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Abbreviations: NBT, nitro blue tetrazolium; BHA, butylated hydroxyanisole; THF, tetrahydrofuran; NaCN, sodium cyanide; EDTA, ethylenediaminetetraacetic acid
Scheme 1. Reagents and Conditions.

(a) Benzyl bromide, K$_2$CO$_3$, acetone, reflux, 4 h, 80%; (b) NaBH$_4$, MeOH, rt, 1 h, 99%; (c) PBr$_3$, THF-benzene, rt, 2 h, triphenylphosphine, benzene, reflux, 3 h, 91%; (d) n-BuLi, 3,5-dimethoxybenzaldehyde, THF, rt, 16 h, 80%; (e) AlCl$_3$, N,N-DMA, CH$_2$Cl$_2$, rt, 2 h, 60%; (f) I$_2$, toluene, reflux, 4 h, 90%.

3,4-Dibenzyloxybenzaldehyde (2). A mixture of 3,4-dihydroxybenzaldehyde (1, 2.0 g, 14.5 mmol), benzyl bromide (5.2 ml, 43.5 mmol), potassium carbonate (9.0 g, 65.6 mmol) and acetone (40 ml) was heated under reflux for 4 h. After completing the reaction, the solid material was filtered off, and the solvent was evaporated. The residue obtained was chromatographed in a silica gel column, using a mixture of petroleum ether and ethyl acetate (95:5) as the eluent to give 2 (4.1 g, 89%), mp 86-88°C (lit.16) mp 89-90°C; IR $\nu_{max}$ (KBr) cm$^{-1}$: 2817, 2726, 1687; NMR $\delta_H$ (90 MHz, CDCl$_3$): 5.20 (4H, s), 6.95 (1H, d, $J$ = 8.5 Hz), 7.01-7.45 (12H, m), 9.70 (1H, s).

3,4-Dibenzyloxybenzyl alcohol (3). To an ice cooled solution of 3,4-dibenzyloxybenzaldehyde (2, 3.5 g, 11.0 mmol) in methanol (50 ml) was added in small portions sodium borohydride (0.62 g, 16.5 mmol), and the mixture was stirred for 1 h at rt. Methanol was distilled off under vacuum, and the residue was diluted with cold water. After acidification with dil. HCl, the solution was extracted with chloroform. The chloroform layer was successively washed with water, 10% sodium bicarbonate and brine, and dried over sodium sulfate. The residue obtained after evaporating the solvent was chromatographed in a silica gel column with petroleum ether-EtOAc (80:20) as the eluent to give 3 (3.5 g, 99%), mp 70-72°C (lit.17) mp 71-72°C; UV $\lambda_{max}$ (MeOH) nm (e): 205 (57,472), 279 (3,184); IR $\nu_{max}$ (neat) cm$^{-1}$: 3281, 1605, 1588, 1514, 1454, 1384, 1255, 1228, 1128, 1003, 738, 695; NMR $\delta_H$ (400 MHz, CDCl$_3$): 4.57 (2H, s), 5.16 (2H, s), 6.87 (1H, dd, $J$ = 1.4 & 8.1 Hz), 6.92 (1H, d, $J$ = 8.1 Hz), 7.00 (1H, d, $J$ = 1.5 Hz), 7.29-7.38 (6H, m), 7.43-7.47 (4H, m).

3,4-Dibenzyloxybenzyltriphenylphosphonium bromide (4). 3,4-Dibenzyloxybenzyl alcohol (3, 2.0 g, 6.25 mmol) in dry tetrahydrofuran (5 ml) and dry benzene (15 ml) were treated, while stirring at 0°C, by the dropwise addition of phosphorus tribromide (0.75 ml, 7.5 mmol) in dry THF (1 ml).
and dry benzene (5 ml). After the reaction mixture had been stirred for 2 h at rt, it was poured into ice cooled water, and the product was extracted with ether. The ethereal extract was washed with brine, dried over sodium sulfate, and the solvent was evaporated. The crude product obtained was dissolved in dry benzene (20 ml) and treated with triphenylphosphine (2.0 g, 7.6 mmol). The reaction mixture was then heated under reflux for 3 h, and cooled and filtered to give 4 (3.7 g, 91%), mp 236–240°C; IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 1064, 960, 814; NMR δ<sub>1</sub> (400 MHz, DMSO-d<sub>6</sub>): 3.78 (6H, s, 3-<b>OCH</b><sub>3</sub>), 6.37 (1H, s, H-4'), 6.72 (2H, d, J = 1.9 Hz, H-2',6'), 6.73 (1H, d, J = 9.0 Hz, H-6), 6.85 (1H, d, J = 16.3 Hz, β-H), 6.88 (1H, d, J = 9.0 Hz, H-5), 6.99 (1H, s, H-3), 7.08 (1H, d, J = 16.3 Hz, α-H), 8.91 (1H, br s, OH), 9.08 (1H, br s, OH); NMR δ<sub>13</sub>C (100 MHz, DMSO-d<sub>6</sub>): 160.63 (C-3',5'), 145.52 (C-1'), 145.24 (C-2'), 139.60 (C-1), 133.93 (C-2), 128.12 (C-4), 125.15 (C-β), 118.96 (C-5), 115.76 (C-6), 113.25 (C-3), 104.05 (C-2',6'), 99.28 (C-4'), 55.15 (3',5'-OCH<sub>3</sub>); MS (ESI, neg. scan): m/z 271 (M-1). Elemental analysis. Found: C, 70.56; H, 5.92%. Calcd. for C<sub>16</sub>H<sub>16</sub>O<sub>4</sub>: C, 70.56%; H, 5.93%.

### Isomerisation of Z-stilbene 5 to E-stilbene 6.

To a solution of Z-stilbene 5 (10 mg) in toluene (5 ml) was added a catalytic amount of iodine, and the mixture was heated to reflux for 4 h. The reaction mixture was diluted with diethyl ether (20 ml) and successively washed with saturated aqueous sodium bisulfite (10 ml) and brine (10 ml). The organic layer was dried over sodium sulfate. The residue obtained after evaporating the solvent was chromatographed in a silica gel column, using hexane-EtOAc (95:5) as the eluent to give E-stilbene 6 (9.0 mg, 90%), mp 180–182°C; IR (neat) ν<sub>max</sub> cm<sup>-1</sup>: 1590, 1510, 1261, 1101, 1019, 958, 772, 735, 695.

### Superoxide free radical-scavenging activity.

The superoxide free radical-scavenging activity was determined by the NBT method. The reaction mixture contained EDTA (6.6 mM), NaN(C3) (3 μg), riboflavin (2 μM), NBT (50 μg), various concentrations of the test drug in ethanol, and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 ml. The optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The percentage inhibition of superoxide radical generation was measured by comparing the absorbance value of the control and that of each test compound. IC<sub>50</sub> values were obtained from a plot of the concentration in μg against the percentage inhibition.

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References


