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Note

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

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4-[2-(3,5-Dimethoxyphenyl)ethenyl]-1,2-benzenediol (7), a stilbene isolated from *Sphaerophysa 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; *Sphaerophysa salsula*; synthesis; antioxidative activity

Free radicals play a major role in the progression of a wide range of pathological disturbances.[1] 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 and antimutagenic activities.[4] 4-[2-(3,5-Dimethoxyphenyl)ethenyl]-1,2-benzenediol (**7**) has recently been isolated from *Sphaerophysa salsula* as a new stilbene.[5] As a part of our continuing study on antioxidative stilbenes,[6,7] we synthesized **7** for the first time and determined its antioxidative activity, the results are being reported in this note.

Sodium borohydride reduction of 3,4-dibenzyloxybenzaldehyde (**2**), which had been obtained from 3,4-dihydroxybenzaldehyde (**1**), in methanol gave 3,4-dibenzyloxybenzyl alcohol (**3**) in a quantitative yield. Reaction of alcohol **3** with phosphorus tribromide and then with triphenyl phosphine gave desired phosphonium salt **4** in a 91% yield. Wittig condensation[8–10] of **4** with 3,5-dimethoxybenzaldehyde in the presence of *n*-butyl lithium gave a mixture of *cis*-stilbene **5** and *trans*-stilbene **6** in an 80% total yield (*E*/*Z*. 1.2:1 by HPLC). Separation of **6** by silica gel column chromatography and subsequent debenzylation with aluminum chloride and *N*,*N*-dimethylaniline[11] gave title compound **7** in a 60% yield (Scheme 1). The spectral data for synthetic **7** agreed well with those of natural **7**. However, attempted debenzylation of *cis*-stilbene **5** resulted in an inseparable mixture of compounds. *Z*-stilbene **5** was efficiently converted to *E*-isomer **6** by heating with a catalytic amount of iodine in refluxing toluene.[12] Thus, **7** was obtained by starting from **1** in five steps in an overall yield of 33%.

In view of the structural similarity of **7** 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 (IC_{50}: 11 μM) in comparison with other known antioxidants: resveratrol (IC_{50}: 360 μM), vitamin E (IC_{50}: 728 μM), vitamin C (IC_{50}: 852 μM) and BHA (butylated hydroxyanisole; IC_{50}: 967 μM). The superior antioxidative activity of new stilbene **7** lends further support to the fact that the catechol system enhances the antioxidative activity.[15]

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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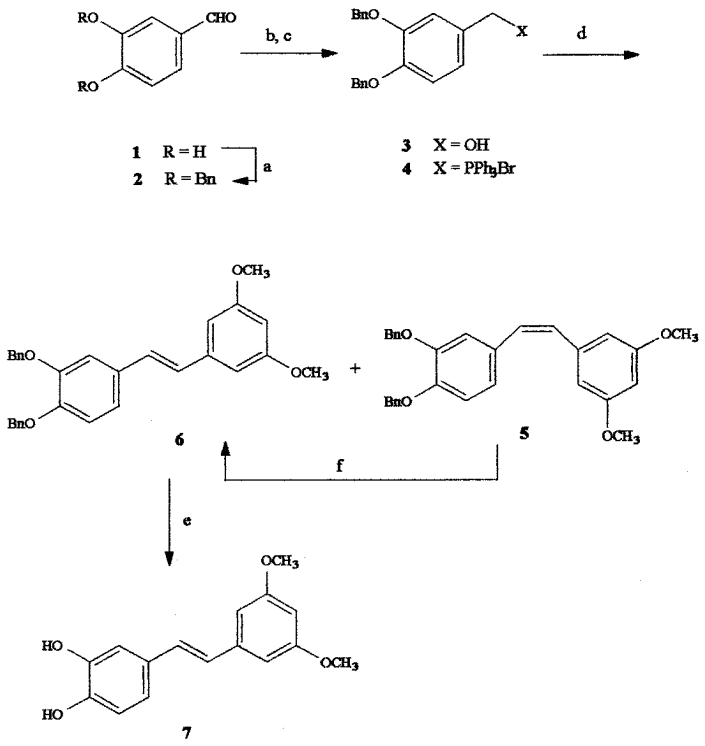
Abbreviations: NBT, nitro blue tetrazolium; BHA, butylated hydroxyanisole; THF, tetrahydrofuran; NaCN, sodium cyanide; EDTA, ethylenediaminetetraacetic acid



Scheme 1. Reagents and Conditions.

(a) Benzyl bromide, K_2CO_3, acetone, reflux, 4 h, 80%; (b) $NaBH_4$, MeOH, rt, 1 h, 99%; (c) PBr_3, THF-benzene, rt, 2 h, triphenyl phosphine, benzene, reflux, 3 h, 91%; (d) *n*-BuLi, 3,5-dimethoxybenzaldehyde, THF, rt, 16 h, 80%; (e) $AlCl_3$, *N*,*N*-DMA, CH_2Cl_2, rt, 2 h, 60%; (f) I_2, toluene, reflux, 4 h, 90%.

analyzer. ^{1}H-NMR of **2** was run at 90 MHz, **5** at 200 MHz and the remaining compounds at 400 MHz. ^{13}C-NMR (100 MHz) spectra were recorded by a Varian Gemini 400 MHz NMR spectrometer, the values for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). Acme silica gel G and silica gel (100–200 mesh) were respectively used for analytical TLC and column chromatography. HPLC was recorded by a Shimadzu SCL-10A instrument under the following conditions: column, Alltima C18; flow rate, 1 ml/min; detection at 324 nm; mobile phase, 0.1% phosphoric acid:acetonitrile (5:95, v/v); retention time for **6**, 4.816 min. and for **5**, 5.013 min.

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 ν_{max} (KBr) cm^{-1}: 2817, 2726, 1687; NMR δ_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 λ_{max} (MeOH) nm (ε): 205 (57,472), 279 (3,184); IR ν_{max} (neat) cm^{-1}: 3281, 1605, 1588, 1514, 1454, 1384, 1255, 1228, 1128, 1003, 738, 695; NMR δ_H (400 MHz, $CDCl_3$): 4.57 (2H, s), 5.16 (2H, s), 5.17 (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 triphenyl phosphine (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_{max} cm^{-1}: 1603, 1587, 1515, 1436, 1266, 1138, 1111, 1017, 742, 694.

5-[2-(3,4-Dibenzyloxyphenyl)ethenyl]-1,3-dimethoxybenzene (5 and 6). To a solution of 3,4-dibenzyloxybenzyltriphenylphosphonium bromide (**4**, 2.52 g, 3.9 mmol) in THF (15 ml) was added *n*-butyl lithium (3 ml, 4.8 mmol, 1.6 M in hexane) at 0°C, and the mixture stirred for 90 min. A solution of 3,5-dimethoxybenzaldehyde (0.5 g, 3.0 mmol) in THF (5 ml) was added to the reaction mixture, which was allowed to warm to rt and further stirred for 16 h. After the usual workup, the residue (*E*/*Z*. 1.2:1 by HPLC) was chromatographed in a silica gel column using a mixture of pet. ether and ethyl acetate (95:5) as the eluent to give **5** (*Z*-isomer, 500 mg, 38%) as an oil; UV λ_{max} (MeOH) nm (ε): 206 (42,180), 280 (26, 243); IR(neat) ν_{max} cm^{-1}: 1590, 1510, 1259, 1204, 1155, 1065, 1019, 868, 737, 696; NMR δ_H (200 MHz, $CDCl_3$): 3.64 (6H, s), 4.91 (2H, s), 5.12 (2H, s), 6.31 (1H, t, J = 2.3 Hz), 6.41 (2H, d, J = 2.2 Hz), 6.44 (2H, s), 6.76–6.82 (2H, m), 6.89 (1H, m), 7.30–7.43 (10H, m); MS (ESI, positive scan): m/z 453 (M + 1). Further elution of the column with pet. ether and EtOAc (95:5) as the eluent gave **6** (E-isomer, 550 mg, 42%), mp180–182°C; UV λ_{max} (MeOH) nm (ε): 202 (43,180), 324 (32,253); IR (neat) ν_{max} cm^{-1}: 1590, 1510, 1261, 1151, 1061, 1019, 958, 772, 735, 695; NMR δ_H (400 MHz, $CDCl_3$): 3.83 (6H, s), 5.18 (2H, s), 5.20 (2H, s), 6.38 (1H, t, J = 2.1 Hz), 6.63 (2H, d, J = 2.1 Hz), 6.84 (1H, d, J = 16.2 Hz), 6.92 (1H, d, J = 8.3 Hz), 6.97 (1H, d, J = 16.2 Hz), 7.02 (1H, dd, J = 1.7 & 8.3 Hz), 7.14 (1H, d, J = 1.7 Hz), 7.31–7.40 (6H, m), 7.44–7.49 (4H, m); MS (ESI, positive scan): m/z 453 (M + 1).

4-[2-(3,5-Dimethoxyphenyl)ethenyl]-1,2-benzenediol (7). To a mixture of **6** (*E*-isomer, 400 mg, 0.91 mmol) and *N*,*N*-dimethylaniline (1.0 ml, 8.2 mmol) in dichloromethane (25 ml) was added aluminum chloride (0.73 g, 5.46 mmol) at 0°C, this temperature being maintained for a further 30 min. The reaction mixture was allowed to warm to room temperature and stirring continued for a further 2 h. The reaction mixture was quenched with 1 N HCl (25 ml) and extracted with ethyl acetate (3 × 25 ml). The organic layer was successively washed with sodium carbonate and brine, and dried over sodium sulfate. The residue obtained after removing the solvent was further purified by column chromatography in a silica gel column with a mixture of chloroform-methanol (98:2) as the eluent to give **7** (150 mg, 60%), mp 122–123°C (lit.[5] mp 72–74°C); UV λ_{max} (MeOH) nm (ε): 327 (2,62,325); IR (KBr) ν_{max} cm^{-1}: 3499, 3327, 1599, 1514, 1312, 1282, 1152, 1064, 960, 814; NMR δ_H (400 MHz, DMSO-d_6): 3.78 (6H, s, 3′,5′-OCH_3), 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_C (100 MHz, DMSO-d_6): 160.63 (C-3′,5′), 145.52 (C-1), 145.24 (C-2), 139.60 (C-1′), 129.27 (C-α), 128.62 (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_3); MS (ESI, neg. scan): m/z 271 (M-1). Elemental analysis. Found; C, 70.56; H, 5.93%. Calcd. for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92%.

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_{max} cm^{-1}: 1590, 1510, 1261, 1151, 1061, 1019, 958, 772, 735, 695.

Superoxide free radical-scavenging activity. The superoxide free radical-scavenging activity was determined by the NBT method.[13,14] The reaction mixture contained EDTA (6.6 mM), NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), 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_{50} values were obtained from a plot of the concentration in μg against the percentage inhibition.

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