Synthesis and Biological Activity of Isoamoenylin, a Metabolite of *Dendrobium amoenum*†

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Isoamoenylin (6), a dihydrostilbene from *Dendrobium amoenum*, was synthesised from 3,4,5-trimethoxybenzaldehyde (1) in four steps with an overall yield of 60%. The spectral data for synthetic 6 are in good agreement with those of the natural product. Isoamoenylin showed moderate antioxidative and weak antibacterial activities.

Key words: isoamoenylin; 3-[2-(3,4,5-trimethoxyphenyl)ethyl]phenol; *Dendrobium amoenum*; antioxidative activity

Dihydrostilbenes, a small group of natural products, have been found to exhibit various biological activities such as antimitotic and endogenous growth regulatory. Isoamoenylin 6, a dihydrostilbene has been recently isolated from the orchid, *Dendrobium amoenum* as a new natural product. As a part of our synthetic studies on phenolics, we synthesised isoamoenylin for the first time and the results are reported in this note.

Sodium borohydride reduction of 3,4,5-trimethoxybenzaldehyde (1) in methanol gave the 3,4,5-trimethoxybenzyl alcohol (2) in a quantitative yield. Phosphonate ester 3 was prepared from 2 by treating with phosphorous tribromide and then by triethyl phosphite in an 82% yield. A Wittig-Horner reaction of 3 with 3-benzyloxybenzaldehyde (4), which had been prepared from 3-hydroxybenzaldehyde, resulted in the presence of sodium hydride furnished trans-stilbene 5 in an 80% yield. Hydrogenation of 5 over palladium-charcoal gave 6 in a 93% yield (scheme 1). The spectral data for synthetic 6 agree well with those of natural 6. Thus, 6 was obtained by starting from 1 in four steps with an overall yield of 60%.

In view of the structural similarity of 6 with natural antioxidants, we screened 6 and its precursor 5 for their antioxidative activity by the nitro blue tetrazolium (NBT) method. 5 and 6 showed moderate superoxide-scavenging activity (IC$_{50}$: 133 and 694 μM, respectively) compared to the other known antioxidants, vitamin E (IC$_{50}$: 728 μM), vitamin C (IC$_{50}$: 852 μM) and BHA (butylated hydroxyanisole; IC$_{50}$: 967 μM).

The antibacterial activity of 6 was determined by the agar cup-plate diffusion method. The zone of inhibition (diameter in mm) at a concentration of 200 μg/ml against *Pseudomonas aeruginosa* (Gram −ve), *Escherichia coli* (Gram −ve), *Bacillus subtilis* (Gram +ve), and *Staphylococcus aureus* (Gram +ve), was 8.5, 9.0, 8.5 and 8.5 mm, respectively. 6 did not show any appreciable antibacterial activity. These compounds did not show any significant antifungal activity against *Aspergillus wentii* or *Aspergillus niger*, even at a 500 μg/ml concentration.

**Experimental**

*General.* All solvents were dried and distilled before use, and all reagents were procured from commercial sources and used without purification. Melting point (mp) data were recorded by a V Scientific 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, and mass spectra by a VG Micromass 70–70H mass spectrometer. δ$_{H}$ NMR (400 MHz) spectra were recorded by a Varian Gemini 400 MHz NMR spectrometer, the value for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. All reactions were carried out in an argon atmosphere, except for the hydrogenation reaction. Acme silica gel G and silica gel (100–200 mesh) were used for analytical TLC and column chromatography, respectively.

3,4,5-trimethoxybenzyl alcohol (2). To an ice cold solution of 3,4,5-trimethoxybenzaldehyde (5 g, 25.5 mmol) in methanol (50 ml) was added in small portions sodium borohydride (1.5 g, 38 mmol), and

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**Abbreviations:** NBT, nitro blue tetrazolium; BHA, butylated hydroxyanisole; THF, tetrahydrofuran; NaCN, sodium cyanide; EDTA, ethylenediaminetetraacetic acid
the mixture was stirred for 1 h at rt. Methanol was distilled off under vacuum, and the product 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 evaporation of the solvent was chromatographed in a silica gel column with petroleum ether-EtOAc (80:20) as the eluent to give 3 (6.5 g, 82%) as an oil.

**Diethyl 3,4,5-trimethoxybenzyl phosphonate (3).** Alcohol 2 (5 g, 25.3 mmol) in THF (5 ml) and benzene (15 ml) was treated with a solution of phosphorous tribromide (3.2 ml, 33 mmol) in THF (1 ml) and benzene (5 ml) while stirring at 0°C. The reaction mixture was allowed to warm to room temperature and left to stand for 2 h. The reaction mixture was then poured into ice-cooled water, and the product was extracted three times with ether. The combined ethereal extract was washed with brine, dried over Na₂SO₄, and the solvent was evaporated. The crude product obtained was heated with triethyl phosphite (6.5 ml, 40 mmol) at 140–150°C for 3 h. The excess triethyl phosphite was removed under vacuum, and the residue was chromatographed in a silica gel column with petroleum ether-EtOAc (1:1) as the eluent to give 3 (6.5 g, 82%) as an oil.

**3-Benzoxybenzaldehyde (4).** A mixture of 3-hydroxybenzaldehyde (1.5 g, 12.3 mmol), benzyl bromide (1.8 ml, 15 mmol), potassium carbonate (3.4 g, 25 mmol) and acetone (25 ml) was heated under reflux for 2 h. After completing the reaction, the solid was filtered off and the solvent was evaporated. The residue obtained was chromatographed in a silica gel column with a mixture of petroleum ether and ethyl acetate (95:5) as the eluent to give 4 (2.2 g, 85%), mp 52–54°C (lit. mp 51–52°C); IR ν_max (neat) cm⁻¹: 3407, 1594, 1506, 1237, 1127, 1006, 828, 774.

**1-(3-Benzxyoxyphenyl)-2-(3,4,5-trimethoxyphenyl) ethylene (5).** To an ice-cooled solution of sodium hydride (0.38 g, 8 mmol) in THF (10 ml) was slowly added phosphonate ester 3 (2.1 g, 6.6 mmol) in THF (5 ml) over 15 min. The solution temperature was allowed to rise to rt, and the mixture stirred for 15 min. After cooling the solution to 5°C, 3-benzyloxybenzaldehyde (4.1 g, 4.7 mmol) in THF (5 ml) was added, and the mixture stirred at the same temperature for 2 h and then at rt for a further 2 h. Methanol (2 ml) was added to the reaction mixture to destroy the excess sodium hydride, before it was diluted with ice-cooled water and acidified with dil. HCl. The
acidic solution was extracted with chloroform, and the organic layer was successively washed with water, 10% sodium bicarbonate and brine, and finally dried over sodium sulfate. The residue obtained was chromatographed in a silica gel column with a mixture of petroleum ether and ethyl acetate (90:10) as the eluant to give 5 (1.4 g, 80%), mp 90–92°; UV $\lambda_{\text{max}}$ (MeOH) nm (e): 210 (28,600), 320 (29,600); IR $\nu_{\text{max}}$ (neat) cm$^{-1}$: 1582, 1503, 1384, 1127, 1008, 956, 773, 691; NMR $\delta_1$ (CDCl$_3$): 3.87 (3H, s), 3.92 (6H, s), 5.11 (2H, s), 6.74 (2H, s), 6.89 (1H, dd, $J=7.9$ & 2.0 Hz), 6.97 (1H, d, $J=16.2$ Hz), 7.03 (1H, d, $J=16.2$ Hz), 7.12 (1H, d, $J=7.8$ Hz), 7.14 (1H, d, $J=2.1$ Hz), 7.28 (1H, t, $J=7.9$ Hz), 7.32–7.47 (5H, m).

3-[2-(3,4,5-trimethoxyphenyl)ethyl]phenol (6). To a solution of 5 (700 mg) in methanol (30 ml) was added palladium-charcoal (10%, 100 mg), and the reaction mixture was stirred under a hydrogen atmosphere for 2 h. The catalyst was removed by filtration, and the solvent was evaporated. The residue obtained was chromatographed in a silica gel column with petroleum ether: ethyl acetate (90:10) as the eluant to give 6 (500 mg, 93%), mp 88–90° (lit.39 semisolid); UV $\lambda_{\text{max}}$ (MeOH) nm (e): 205 (58,400), 274 (3,080); IR $\nu_{\text{max}}$ (neat) cm$^{-1}$: 3405, 1592, 1507, 1457, 1238, 1126, 1004, 827, 775, 697; NMR $\delta_1$ (CDCl$_3$): 2.85 (4H, br s, 1.2-H), 3.82 (6H, s, Ar-OME), 3.83 (3H, s, Ar-OME), 5.04–5.05 (1H, s, Ar-CH$_2$), 6.36 (2H, s, 2',6'-H), 6.66 (1H, s, 2'-H), 6.67 (1H, d, $J=7.8$ Hz, 4'-H), 6.76 (1H, d, $J=7.8$ Hz, 6'-H), 7.15 (1H, t, $J=7.6$ Hz, 5'-H); EIMS m/z (rel. int.): 289 (M+1, 5), 288 (M', 24), 182 (14), 181 (100), 107 (9) and 77 (9).

Antioxidative activity

Superoxide free radical-scavenging activity. The superoxide free radical-scavenging activity was determined by the NBT method.10,11 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. 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 values of the control and those of the test compounds. IC$_{50}$ values were obtained from a plot of the concentration in μg against the percentage inhibition.

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References


