Synthesis and antioxidant activity of hispolon, a yellow pigment from *Inonotus hispidius*

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Hispollon 5 has been synthesised from veratraldehyde 1, in four steps, with an overall yield of 23%. The spectral data of synthetic 5 are in agreement with those of natural 5. Hispolon shows strong antioxidant and weak antibacterial activities.

Hispollon 5 was isolated, recently, as yellow pigment from a parasitic fungus, *Inonotus hispidius* (Bull. Ex Fr.) Karst. (Basidiomycetes) during bioactivity guided isolation of the extract which showed immuno-modulatory and antiviral activity. Due to our interest on diarylheptanoids, we have synthesised 5, for the first time and the results are reported in this paper.

Knoevenagel-Doebner condensation of veratraldehyde 1 with malonic acid gave 3,4-dimethoxycinnamic acid 2 in 80% yield. The reaction of 3,4-dimethoxycinnamoyl chloride, prepared from 2, was treated with ethyl acetocetate in the presence of NaH to give ethyl (4E)-2-acetyl-5-(3,4-dimethoxyphenyl)-3-oxo-4-pentenoate 3 in 78% yield. Decarboxylation of 3 using DMSO/NaCl afforded 4-hydroxy-6-(3,4-dimethoxyphenyl)-hexa-3,5-diene-2-one 4 in 61% yield. Demethylation of 4 using BBr3 gave the title compound 5 in 60% yield (Scheme 1).

The spectral data of synthetic 5 agree well with those reported for natural 5. Thus, 5 was obtained starting from veratraldehyde 1, in four steps with an overall yield of 23%.

As caffeic acid and its derivatives show strong antioxidant activity, and in view of structural similarity of 5 with caffeic acid derivatives, we have screened 5 for antioxidant activity by the nitroblue tetrazolium (NBT) reduction method. 5 showed strong superoxide scavenging activity (IC50: 6 µg/mL) compared to other known antioxidants, vitamin E (IC50: >2000 µg/mL), BHT (butylated hydroxytoluene, IC50: 62 µg/mL) and BHA (butylated hydroxylanisole, IC50: 240 µg/mL). 4, in which no free hydroxyl groups are present on the aromatic ring, showed as anticipated diminished antioxidant activity (IC50: 70 µg/mL).

![Scheme 1](image-url)

(i) Malonic acid, pyridine, piperidine, Δ, 1 hr, 80%; (ii) SOCl2, reflux, 2hr, NaH, CH2(COCH)COOEt, THF, r, 4hr, 70%; (iii) DMSO/NaCl, H2O, 150-160°C, 4hr, 61%; (iv) BBr3, CH2Cl2, r, 12 hr, 60%

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Compounds 4 and 5 showed weak antibacterial activity against Gram +ve (Bacillus subtilis, and Bacillus Pumilis) and Gram -ve (Pseudomonas aeruginosa) bacteria at a concentration of 50 μg/mL (Zone of inhibition of about 8.0 mm in diameter).

Experimental Section

Melting points were recorded on a V Scientific melting point apparatus, in open capillaries and are uncorrected. UV spectra were recorded on a Shimadzu UV-190 Spectrophotometer, IR spectra on a Perkin-Elmer BX1 FTIR Spectrophotometer, 1H NMR(90 MHz), and 13C NMR (22.5 MHz) spectra on a Jeol JNM EX 1080, NMR(90 MHz) and UV spectra were recorded on a Shimadzu UV-190 Spectrophotometer, and IR spectra on a Shimadzu UV-190 Spectrophotometer.

The reaction mixture at 10 mL was added dropwise to the reaction mixture at 100°C, for 4 hr. The reaction mixture was quenched with water (100 mL) and evaporated and filtered. Solvent was evaporated. The residue was chromatographed over silica gel column eluting with hexane-ethyl acetate (96 : 4) to give 4 (800 mg, 61%), m.p. 890°C; UV (MeOH) (log ε): 206 (4.26), 369 (4.49); NMR (CDCl3, 90 MHz): δ 2.14 (3H, s, H-1), 3: (6H, s, 2 × Ar-OCH3), 5.64 (1H, s, H-3), 6.33 (1H, J = 16.0 Hz, H-5), 6.85 (1H, d, J = 8.4 Hz, H-6), 7: (1H, s, H-2'), 7.1 (1H, d, J = 8.4 Hz, H-5'), 7.54 (1H, d, J = 16.0 Hz, H-6); 13C NMR (CDCl3), 22.5 MHz: 196.7 (C-2), 177.8 (C-4), 150.9 (C-4'), 149.2 (C-2), 139.7 (C-6), 128.0 (C-1'), 122.2 (C-6'), 120.6 (C-11.2) (C-5'), 109.8 (C-2'), 100.6 (C-3'), 55.8 (C-OMe), 26.6 (C-1).

Hispolon [4-hydroxy-6-(3,4-dihydroxyphenyl)hexa-3,5-dien-2-one] 5, To a cold stirred solution 4 (500 mg, 2.01 mmole) in CH2Cl2 at -10°C was added boron tribromide (8.0 mL, 8.1 mmole, 1 solution in dichloromethane). The reaction mixture was slowly brought to room temperature and stirred for 12 hr. The solvent was evaporated and added hydrochloric acid (2N, 20 mL). The solution was extracted with ethyl acetate (2 × 30 mL) and dried over sodium sulfate. Evaporation of the solution followed by chromatography of the residue on silica gel column using chloroform-methanol (99:1 as eluent, furnished hispolon 5 (265 mg, 60%), m.p. 135-37°C (Lit. mp 137°C); UV (MeOH) (log ε): 208 (4.19), 371 (4.52); IR (KBr): 3376, 3122, 1640-300 cm-1; 1H NMR (d6-acetone, 90 MHz): 5: 2.1 (3H, s, H-1, signal merged with solvent signal), 5: (1H, s, H-3), 6.45 (1H, d, J = 15.8 Hz, H-5), 6: (1H, d, J = 8.4 Hz, H-5'), 7.04 (1H, dd, J = 8.1, 2 Hz, H-6), 7.15 (1H, d, J = 2.0 Hz, H-2'), 7.48 (1H, d, J = 15.8 Hz, H-6), 8.06 (1H, brs, Ar-OH), 8: (1H, brs, Ar-OH); 13C NMR (d6-acetone, 22.5 MHz): δ 198.1 (C-2), 179.9 (C-4), 149.0 (C-4'), 146.8 (C'), 141.4 (C-6), 128.9 (C-1'), 122.9 (C-6'), 121.3 (C'), 116.9 (C-5'), 115.6 (C-2'), 101.6 (C-3), 27.0 (C). EIMS m/z (%): 220 (M+, 31), 202 (43), 187 (26), 182 (45), 181 (46), 177 (26), 167 (65), 163 (43), 15 (20), 145 (16), 135 (26), 131 (21), 91 (60), 77 (40), 44 (100).
Antioxidant activity
Inhibition of superoxide scavenging was determined by the nitroblue tetrazolium (NBT) reduction method. The reaction mixture comprises of EDTA (6 μM) containing 3 μg NaCN, riboflavin (2 μM), NBT (50 μM), various concentrations of the 5 (or 4), in ethanol and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 530 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and compound treated tubes.

Antibacterial activity
Hispolon 5 and its dimethyl ether 4 were screened for their antibacterial activity by the agar cup-plate diffusion method. The zone of inhibition (diameter in mm) at a concentration of 50 μg/mL in methanol for 4 and 5 against Pseudomonas aeruginosa (Gram –ve, 8.0 and 8.0 mm), Escherichia coli (Gram –ve, 0 and 0 mm), Bacillus subtilis (Gram +ve, 8.0 and 8.5 mm) and Bacillus pumilis (Gram +ve, 8.5 and 9.0 mm), respectively.

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References