Synthesis and biological activities of prenylated tyrosine derivatives, the metabolites of *Pithomyces ellis* \(^1\)

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2-[4-(2E)-3-Methylbut-2-enyloxy)phenyl]ethan-1-ol \(3\) and 3-[4-(2E)-3-methylbut-2-enyloxy)phenyl]-2-(acetylamino)propanoic acid \(8\) have been synthesized from 4-hydroxyphenyacetic acid \(1\), l-tyrosine \(4\), respectively, with an overall yield of 55% and 37%. The spectral data of synthetic \(3\) and \(8\) are in good agreement with those of natural \(3\) and \(8\), respectively. These synthetic \(3\) and \(8\) show significant brine shrimp lethality, but weak antioxidant and antibacterial activities.

**Keywords:** Prenylated tyrosine, metabolites, *Pithomyces ellis*, 4-hydroxyphenyacetic acid, antioxidant activity, antibacterial activity

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L-tyrosine is a non-essential amino acid for human development, precursor for the synthesis of thyroid hormones and select neurotransmitters, such as dopamine and norepinephrine. It is considered to be essential for the brain. The tyrosine derivatives have been marketed as anti-parkinsonian drugs (levodopa), antispasmodic drugs (tioproamide), platelet aggregation inhibitors or receptor antagonists (tirafibran hydrochloride) and as pancreas function diagnostic compounds (bentiromide). 2-[4-(2E)-3-Methylbut-2-enyloxy)phenyl]ethan-1-ol \(3\) and 3-[4-(2E)-3-methylbut-2-enyloxy)phenyl]-2-(acetylamino)propanoic acid \(8\) were isolated, recently as novel 5-HT receptor ligand from the fungi, *Pithomyces ellis* \(^2\). Due to our interest on simple antioxidative natural products \(^3,4\), we have synthesized compounds \(3\) and \(8\) for the first time and the results are reported in this note.

**Results and Discussion**

Reduction of 4-hydroxyphenyacetic acid \(1\) by the in situ generated diborane using sodium borohydride and iodine \(^5\) gave 4-(2-hydroxyethyl) phenol \(2\) in 88% yield. Compound \(2\) was prenylated using prenyl bromide in the presence of potassium carbonate to give the desired 2-[4-(2E)-3-methylbut-2-enyloxy)phenyl]ethan-1-ol \(3\) in 62% yield (Scheme 1). The spectral data of synthetic \(3\) agree well with those reported for natural \(3\). Thus, \(3\) was obtained starting from 4-hydroxyphenyacetic acid \(1\), in two steps with an overall yield of 55%. Compound \(2\) was isolated earlier as a natural product from blue stain fungi \(^6\).

Esterification of l-tyrosine \(4\) using methanolic HCl followed by acylation of amine using acetyl chloride furnished N-acetyl-l-tyrosine methyl ester \(6\) in good

![Scheme 1](image)

Reagents and conditions: (i) NaBH\(_4\), \(\text{L}\), THF, reflux, 2 hr, 88%
(ii) prenyl bromide, \(\text{K}_2\text{CO}_3\), acetone, reflux, 4 hr, 62%.

\(^{1}\) Laila Impex Communication # 42
yield. The phenolic group was prenylated using prenyl bromide in presence of potassium carbonate followed by hydrolysis of the ester function using methanolic potassium hydroxide to give the desired 3-[4-(2E)-3-methylbut-2-enloyloxy]phenyl]-2-(acetylamino) propanoic acid 8 (Scheme II). The spectral data of synthetic 8 agree well with those reported for natural 8. Thus, 8 was obtained starting from L-tyrosine 4, in four steps with an overall yield of 37%.

Compounds 3 and 8 were screened for their cytotoxic activity by the brine shrimp lethality test (BST) and antioxidant activity by the nitro blue tetrazolium (NBT) method. The synthetic 3 and 8 showed significant brine shrimp lethality (IC50: 22 µg and 50 µg respectively). Compound 3 displayed lower superoxide scavenging activity (IC50: 1505 µM) compared to the other known antioxidants, vitamin E (IC50: 728 µM), vitamin C (IC50: 852 µM) and BHA (butylated hydroxyanisole; IC50: 967 µM). The compound 8 did not show any appreciable superoxide scavenging activity even at 500 µg/mL concentration.

Antibacterial activity of compounds 3 and 8 was determined by the agar cup-plate diffusion method. The zone of inhibition (diameter in mm) at a concentration of 500 µg/mL for 3 and 8 against Pseudomonas aeruginosa were 8.5 and 0.0 mm, against Escherichia coli were 9.0 and 0.0 mm, against Bacillus subtilis were 9.5 and 8.5 mm and against Staphylococcus aureus were 9.0 and 0.0 mm, respectively.

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 (400 MHz) and 13C NMR (100 MHz) spectra on Varian Gemini 400 MHz NMR spectrometer; and mass spectra on an Agilent 1100 LCMS instrument. Acme silica gel G and silica gel (100-200 mesh) were used for analytical TLC and column chromatography, respectively.

4-(2-Hydroxyethyl)phenol 2. To an ice cold solution (0-5°C) of sodium borohydride (1.8 g, 49.3 mmoles) in THF (20 mL) was added a solution of iodine (1.6 g, 13.1 mmoles) in THF (5 mL) during 1.5 hr. To this, 4-hydroxyphenylacetic acid 1 (0.5 g, 3.3 mmoles) in THF (5 mL) was added and the solution was stirred at rt for 1 hr and refluxed for 2 hr. The cooled reaction mixture was acidified with dil HCl and the solution was extracted with ethyl acetate. The combined ethyl acetate layer was washed with water, brine and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using chloroform-methanol (95:5) as eluent to get tyrosol 2 (0.4 g, 88%). Recrystallisation of 2 from chloroform-hexane gave a white solid, m.p. 90-92 ºC (lit.6 m.p. 91-92 ºC); IR (neat): 3394, 3154, 1604, 1235, 1052, 1014, 819 cm⁻¹; 1H NMR (CDCl3): δ 7.10 (2H, d, J = 8.41 Hz), 6.78 (2H, d, J = 8.41 Hz), 4.81 (1H, s), 3.83 (2H, br s), 2.81 (2H, t, J = 6.5 Hz); LCMS (negative ion mode): m/z 137 (M–H)⁻.

2-[4-(2E)-3-M ethylbut-2-enloyloxy]phenyl]-ethan-1-ol 3. A mixture of tyrosol 2 (0.25 g, 1.8 mmoles), prenyl bromide (0.41 g, 2.7 mmoles), potassium carbonate (0.75 g, 5.4 mmoles) and acetone (40 mL) was heated under reflux for 4 hr. After completion of the reaction, the solid was filtered off and the solvent was evaporated. The residue obtained was chromatographed over a silica gel column with a
mixture of petroleum ether-ethyl acetate (9:1) as the eluent to give 3 as an oil (230 mg, 62%) (lit.2 oil); UV (MeOH) (log ε): 280 (3.19), 226 (3.83), 205 (4.25) nm; IR (neat): 3316, 3272, 2927, 1607, 1260, 1241, 1219, 1068, 1014, 818, 795 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.09 (2H, d, \(J = 8.6\) Hz, H-4,4'), 6.82 (2H, d, \(J = 8.6\) Hz, H-5,5'), 5.4-5.5 (1H, m, H-8), 4.46 (2H, d, \(J = 6.6\) Hz, H-7), 3.79 (2H, t, \(J = 6.6\) Hz, H-1), 2.78 (2H, t, \(J = 6.3\) Hz, H-2), 1.79 (3H, s, H-10), 1.74 (3H, s, H-11); \(^13\)C NMR (CDCl\(_3\)): \(\delta\) 157.7 (C-6), 137.5 (C-9), 130.2 (C-3), 129.9 (C-4,4'), 120.2 (C-8), 114.8 (C-5,5'), 64.7 (C-7), 63.8 (C-1), 38.43 (C-2), 25.9 (C-10), 18.3 (C-11); LCMS (negative ion mode): m/z 205 (M–H)\(^{-}\).

**L-Tyrosine methyl ester** 5. A solution of L-tyrosine 4 (3.6 g) in methanolic HCl (60 mL, 2.5 N) was refluxed for 9 h and the solution was concentrated in vacuo. The residue was dissolved in methanol (40 mL), excess of triethylamine (4.0 mL) was added (to neutralise HCl) and stirred for 1 hr at rt. The salt was filtered and the residue was chromatographed over silica gel column using chloroform-methanol (90:10) as eluent to obtain 5 (2.8 g, 72%), m.p. 133-34°C (lit.\(^{10}\) m.p. 135-36°C); IR (KBr): 3469, 3358, 2931, 1744, 1598, 1264, 1176, 1020, 836 cm\(^{-1}\); LCMS (positive ion mode): m/z 196 (M+H\(^{+}\)).

**N-Acetyl-

L-tyrosine methyl ester** 6. To a solution of 5 (2.0 g) in chloroform (150 mL) was added sequentially sodium carbonate (2.0 g) and water (13 mL). After stirring the reaction mixture for 30 min, acetyl chloride (2.0 mL) was added and stirred for further 3 hr at rt. The solid was filtered and the aqueous layer was extracted with ethyl acetate. The combined solid and the residue obtained after evaporation of the solvent from ethyl acetate extract was chromatographed over silica gel column using chloroform-methanol (95:5) as eluent to yield 6 (2.0 g, 82%), m.p. 134-36°C (lit.\(^{11}\) m.p. 136-37 °C); IR (KBr): 3551, 3324, 3158, 1734, 1661, 1285, 1236, 1132, 1047, 827 cm\(^{-1}\); LCMS (positive ion mode): m/z 238 (M+H\(^{+}\)).

**N-(1-[4-(2E)-3-M ethylbut-2-enoxy]phenyl)methyl)-2-oxobutyl|acetamide** 7. To a mixture of 6 (1.2 g, 5.1 mmoles), potassium carbonate (1.4 g, 10.1 mmoles) in acetone (50 mL) was added prenyl bromide (1.13 g, 7.6 mmoles) and the mixture was refluxed for 2 hr. The undissolved material was filtered off and the solvent was evaporated from the filtrate under reduced pressure. The residue obtained was extracted with chloroform and the chloroform layer was washed with brine, dried over sodium sulfate and filtered. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using chloroform-methanol (98:2) as eluent to afford 7 (1.3 g, 84%) as an oil. IR (neat): 3286, 3033, 2928, 1744, 1659, 1236, 1129, 1006, 831 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 6.98 (2H, d, \(J = 8.5\) Hz, H-4,4'), 6.83 (2H, d, \(J = 8.5\) Hz, H-5,5'), 5.78-5.84 (1H, m, NH), 5.45-5.51 (1H, m, H-8), 4.53-4.57 (1H, m, H-1), 4.47 (2H, d, \(J = 6.6\) Hz, H-7), 3.72 (3H, s, COOME), 3.0-3.1 (2H, m, H-2), 1.98 (3H, s, H-14), 1.79 (3H, s, H-10), 1.73 (3H, s, H-11); LCMS (positive ion mode): m/z 306 (M+H\(^{+}\)).

3-[4-(2E)-3-M ethylbut-2-enoxy)phenyl]-2-(acetylamo)propanoic acid 8. A solution of 7 (1.0 g) in methanolic KOH (5%, 20 mL) was stirred at rt for 90 min and the solvent was evaporated under reduced pressure. The residue was diluted with ice cooled water and acidified with dil. HCl. The precipitated solid was filtered and recrystallised from chloroform-hexane to obtain 8 (720 mg, 75%) as white solid, m.p. 147-48°C (lit.\(^{2}\) white solid, m.p. not reported); UV (MeOH) (log ε): 277 (4.10), 217 (4.20), 205 (4.22) nm; IR (KBr): 3343, 2920, 1699, 1621, 1244, 1006 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.07 (2H, d, \(J = 8.5\) Hz, H-4,4'), 6.85 (2H, d, \(J = 8.5\) Hz, H-5,5'), 5.84-5.91 (1H, m, NH), 5.45-5.51 (1H, m, H-8), 4.79-4.84 (1H, m, H-1), 4.48 (2H, d, \(J = 6.6\) Hz, H-7), 3.05-3.19 (2H, m, H-2), 2.0 (3H, s, H-14), 1.79 (3H, s, H-10), 1.74 (3H, s, H-11); \(^13\)C NMR (CDCl\(_3\)): \(\delta\) 174.2 (C-13), 171.2 (C-12), 158.1 (C-6), 137.9 (C-9), 130.3 (C-4), 127.6 (C-3), 119.7 (C-8), 114.8 (C-5), 64.8 (C-7), 53.5 (C-2), 36.4 (C-1), 25.7 (C-10), 22.7 (C-14), 18.1 (C-11); LCMS (negative ion mode): m/z 290 (M–H)\(^{-}\).

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**References**