Oxidation of Tyrosine Diketopiperazine to DOPA Diketopiperazine with Tyrosine Hydroxylase†

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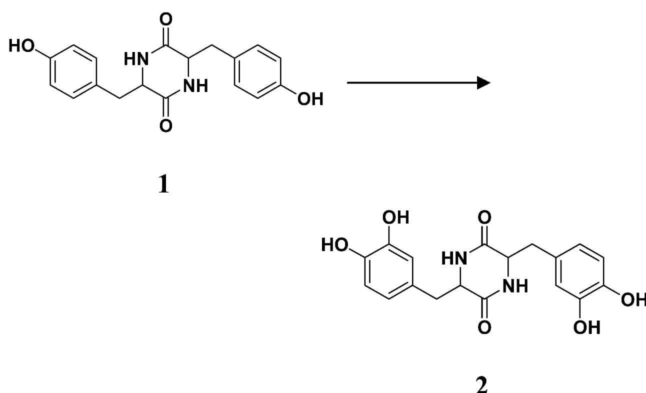
The diketopiperazine of DOPA was synthesized in high yield from the diketopiperazine of tyrosine using PC12 cell lysate, which expresses high levels of tyrosine hydroxylase. This represents the first use of this enzyme to prepare DOPA-containing peptides.

In a recent report, we demonstrated that diketopiperazines (DKP) of tyrosine (**1**) and 3,4-dihydroxyphenylalanine (DOPA) (**2**) are intermediates in the biosynthesis of ecteinascidin 743 (ET-743).[1] The ecteinascidins are a family of tetrahydroisoquinoline alkaloids isolated from the tunicate *Ecteinascidia turbinata*.[2] At nanomolar concentrations, ET-743 is active against a variety of solid tumor cell lines, including melanoma, non-small cell lung carcinoma (NSCLC), and ovarian and colon cancer cell lines,[3] and against a variety of surgically derived human tumor specimens growing in primary cultures.[4] Furthermore, ET-743 is very active in vivo against several types of human solid tumor xenografts.[5] ET-743 bears significant structural homology to the saframycin family of antibiotics and other related compounds.[6]



Since **2** is a key intermediate in ecteinascidin biosynthesis and since the chemical synthesis of DOPA derivatives is problematic,[7] we investigated the use of tyrosine hydroxylase as a tool to oxidize **1** to **2**. Tyrosine hydroxylase (EC 1.14.16.2) catalyzes the rate-limiting step in catecholamine biosynthesis,[8] specifically the hydroxylation of tyrosine to DOPA.[8] The rat pheochromocytoma PC12 cell line established by Greene and Tischler[9] expresses high levels of tyrosine hydroxylase and thus provides a convenient source of this enzyme.

Experimental Section

General Experimental Procedures. PC12 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% horse serum (HS), 5% fetal calf serum (FCS), and 100 μg/mL penicillin and streptomycin to confluency. The cells were harvested using trypsin-EDTA and collected by centrifugation at 800*g* for 5 min. The cell pellet was washed, resuspended in phosphate-buffered saline (PBS) (pH 7.4), and disrupted using a sonicator. Cell debris was removed by centrifugation for 20 min, and the resulting lysate was stored at −80 °C. To confirm the activity of the tyrosine hydroxylase in this preparation, DOPA was synthesized from L-tyrosine in 95% yield according to the protocol of Nagatsu et al.[10]

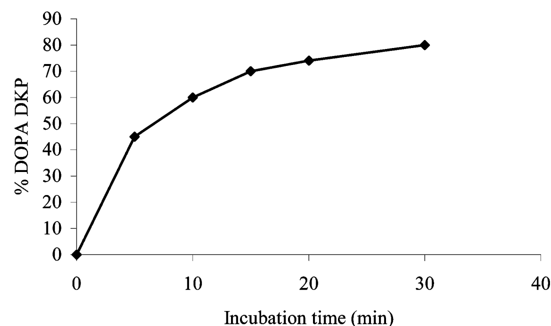


Figure 1. Time course evaluation of the oxidation of **1** to **2**.

The oxidation of diketopiperazine **1** was conducted using the cell lysate described above. The incubation mixture consisted of the following components: 10 μL of 1 M acetate buffer at pH 6.0, 20 μL of 1 mM of **1** in 0.01 M HCl, 10 μL of 10 mM 6-methyl-5,6,7,8-tetrahydropterin in 1 M 2-mercaptoethanol, 1 mL of PBS buffer containing enzyme, 10 μL of 1 mg/mL catalase. The incubation was performed at 37 °C for 30 min, and the reaction terminated by adding 600 μL of 0.5 M perchloric acid at 0 °C. After 10 min, 20 μL of 0.2 M EDTA and 300 μL of 1 M potassium carbonate were added, and the mixture was centrifuged at 1600*g* for 10 min at 4 °C. The clear supernatant was subjected to reversed-phase HPLC using a mobile phase of 90% H_2O (0.1% TFA)/10% CH_3CN. The production of **2** was confirmed by ESIMS and by comparison of HPLC retention times with that of an authentic standard.[11] HPLC analysis of reaction mixtures quenched at various time intervals indicated that a yield of 80% is achieved in 30 min (Figure 1). Interestingly, there was no evidence for the formation of the intermediate oxidation product in the LC-MS analysis.

This production of the DKP of DOPA (**2**) provides an efficient preparation of a key intermediate in ecteinascidin biosynthesis. Further, it represents the first use of tyrosine hydroxylase to generate DOPA-containing peptides. Our observation of the production of **2** from **1** suggests that this system may be applied to the synthesis of a variety of peptides with a DOPA residue.

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(11) An authentic sample of **2** was obtained by an alternative synthesis and confirmed by ^{1}H NMR, ^{13}C NMR, and ESIMS according to our previous report (see ref 1).

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