Note

A Convenient Method for the Preparation of *N*-β-Alanyldopamine as a Substrate of Phenoloxidase

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N-β-Alanyldopamine (NBAD) has attracted a great deal of interest because of its significance in catecholamine metabolism in insects with which phenoloxidase is concerned.[1] Kramer *et al.* synthesized NBAD by coupling the acid component of *N*-β-alanine with the amine component of dopamine in aqueous solution.[2] Their method, however, required several chromatographic steps to purify the synthetic compound. We recently found a method to prepare NBAD more conveniently. This report describes a new synthetic method of NBAD and the oxidation of NBAD by housefly phenoloxidase using this synthetic compound.

Phenoloxidase (EC 1.14.18.1 in part) was prepared by the method of Shimoda *et al.*[3] from housefly (*Musca domestica vicina*) pupae three days after puparium formation and its activity was measured as described previously.[4] Boc-BA-ONSu was synthesized by the method of Smirnov *et al.*[5] NBAD was synthesized as follows:

Boc-BAD (**1**). To a solution of dopamine hydrochloride (984 mg, 5 mmol) in dimethylformamide (5 ml) Boc-BA-ONSu (1.43 g, 5 mmol) and triethylamine (1.28 ml, 10 mmol) were added, and the mixture was stirred at room temperature for 2 hr. After removal of the precipitate by filtration, the filtrate was evaporated *in vacuo*, and the residue was dissolved in EtOAc. The solution was washed successively with 10% citric acid and water, dried over Na_2SO_4, and evaporated. The residue was recrystallized from 2-propanol: yield 1.2 g (70%), mp 154–157°C; *Rf*=0.72 on silica gel ($CHCl_3$–MeOH–AcOH, 50:10:2). *Anal.* Found. C, 59.11; H, 7.46; N, 8.63%. Calcd. for $C_{16}H_{24}N_2O_5$: C, 59.24; H, 7.46; N, 8.63%.

NBAD·HCl (**2**). A solution of **1** (1.1 g, 3 mmol) in 4 N HCl in dioxane (4 ml, 16 mmol) was left at room temperature for 3 hr and evaporated *in vacuo*. The crystals formed were washed with ether: yield, 850 mg (95%), mp 189–191 C; *Rf*=0.62 on silica gel (BuOH–AcOH–pyridine–H_2O, 4:1:1:2). *Anal.* Found: C, 49.87; H, 6.52; N, 10.48%. Calcd. for $C_{11}H_{16}O_3N_2 \cdot HCl$: C, 50.67; H, 6.57; N, 10.57%. ^1H NMR (in D_2O): δ=2.59 (2H, t, J=6.88 Hz), 2.70 (2H, t, J=6.72 Hz), 3.18 (2H, t, J=6.88 Hz), 3.41 (2H, t, J=6.72 Hz), 6.71 (1H, d, J=8.08 Hz), 6.80 (1H, s), and 6.86 (1H, d, J=8.08 Hz).

As described above, NBAD·HCl, **2**, was obtained conveniently in high quality as examined by elemental analysis, thin layer chromatography, and NMR spectrum. It is known that dopamine is readily oxidized in alkaline solutions. However, when coupling of Boc-BA-ONSu with dopamine was done under the conditions established here, **1** was obtained without formation of the oxidized derivative. **2** was obtained as crystals during treatment of **1** with HCl in dioxane. Thus, in our method the reactions were done in the oraganic solvents to obtain the final product **2** in a fairly high yield.

When **2** was incubated with phenoloxidase purified from housefly pupae at pH 6.5 and 20°C, the absorbance at 390 nm of the reaction mixture increased with the progress of the incubation time, owing to the oxidation of NBAD. From these results, it is suggested that this method can be used advantageously for synthesis of NBAD. Kinetic studies on phenoloxidases from houseflies and other species of insects are being done with the synthetic NBAD.

References

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Abbreviations: NBAD, *N*-β-alanyldopamine; Boc-BA-ONSu, *t*-butyloxycarbonyl-β-alanine-*N*-hydroxysuccinimide ester; Boc-BAD; *t*-butyloxycarbonyl-β-alanyldopamine.