Synthesis of 2-Amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine

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2-Amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine (2), a precursor of Watasenia preluciferin (coelenterazine) (1), is widely distributed in marine bioluminescent animals. It was prepared from *p*-hydroxyphenylglyoxal aldoxime (5) in two steps; by condensation with α-aminophenylpropiononitrile in the presence of $TiCl_4$ in pyridine, followed by reduction of the resulting *N*-oxide (6) with Zn–AcOH in CH_2Cl_2 and produced 2, with an 89% overall yield. This procedure was linked with the facile one-step preluciferin synthesis reported in the previous paper. Thus, Watasenia preluciferin (1), frequently required for various chemiluminescent and bioluminescent studies, was coveniently synthesized in three steps from 5, with a 56% overall yield, overcoming the difficulty of obtaining it from natural sources.

Key words 2-aminopyrazine derivative; precursor; Watasenia preluciferin; bioluminescent compound

8-Benzyl-2-(*p*-hydroxybenzyl)-6-(*p*-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (**1**) from the liver of the squid, *Watasenia scintillans* (Japanese name: Hotaruika),[1)] was first isolated in 1975 and termed Watasenia preluciferin. It plays a key role in the light emission systems of various marine luminescent organisms[2a)] such as squids,[2b)] shrimps,[2c)] coelenterates[2d)] and fishes.[2e)] All specimens contained Watasenia preluciferin (coelenterazine) (**1**) or its derivatives together with 2-amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine (**2**) and/or its derivatives as the precursor or the light emitter of their bioluminescent compounds. Compound **2** was first isolated as the single chromophor from the jellyfish, Aequorea, by Shimomura and Johnson[3)] in 1972 and it was subsequently synthesized by Goto and colleagues[4)] from *p*-methoxyphenylglyoxal aldoxime (**3**) *via* *N*-oxide (**4**) in three steps. Watasenia preluciferin (**1**) was prepared from its precursor (**2**) by cyclization with *p*-hydroxybenzylglyoxal in an acidic medium and was converted to various bioluminescent compounds.[2b,d,e)] Recently, the authors found that *p*-hydroxyphenylpyruvic acid reacted with **2**, without any reductive treatment, to give **1** directly in a one batch reaction process.[5)] As part of the work on the simple step preparation of **1**, a more compact synthesis of **2** was examined. Thus, when *p*-hydroxyphenylglyoxal aldoxime (**5**),[6)] in place of **3**, was reacted with α-aminophenylpropiononitrile[7)] in the presence of $TiCl_4$ in pyridine at -5 to 20 °C, it gave *N*-oxide (**6**) in 94% yield. On treating **6** with Zn–AcOH in CH_2Cl_2, *N*-oxide was readily removed to afford the desired precursor (**2**) in 95% yield. Subsequently, **2** was condensed with *p*-hydroxyphenylpyruvic acid, to give **1** (63%) according to the improved method.[5)] With this, the overall yield of the total synthesis of **1** starting from **5** in three steps was 56.26%. Compound **1** and related luminous analogues, frequently required for chemiluminescent and bioluminescent studies, are difficult to obtain from natural sources. The short step synthesis of **1** described herein has made it possible to easily obtain a considerable amount of each compound. In addition, this compact preparative method is generally applicable to the synthesis of a wide range of luminous imidazopyrazinone analogues.[8a—d)]

Experimental

Melting points are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL A-600 (600 MHz) spectrometer with trimethylsilane (TMS) as internal standard.

2-Amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine-1-oxide (6) To a cold solution (-5 °C) of *p*-hydroxyphenylglyoxal aldoxime (**5**)[6)] (330 mg, 2 mmol) and α-aminophenylpropiononitrile hydrochloride[7)] (436 mg, 2.4 mmol) in pyridine (8 ml) was added $TiCl_4$ (1.89 g, 10 mmol) drop-wise with vigorous stirring. The mixture was warmed gradually to 20 °C. On completion of the reaction (*ca.* 30 min, judging from TLC, MeOH–CH_2Cl_2=1 : 10), the mixture was poured into ice-water, 10% HCl (40 ml) was added to the mixture and the separated precipitate was taken up in AcOEt. The AcOEt layer was washed with water and dried over Na_2SO_4. Removal of the solvent left a crystalline solid which was washed with ether to afford **6** (551 mg, 94%), mp 230 °C (decomp). This product was sufficiently pure for further use. A small sample was recrystallized from MeOH to give pale yellow needles, mp 230—233 °C (decomp). ^{1}H-NMR (DMSO-d_6) δ: 4.17 (2H, s), 6.80 (2H, d, J=8.4 Hz), 6.95 (2H, s), 7.20 (1H, t, J=7.6 Hz), 7.30 (2H, t, J=7.6 Hz), 7.35 (2H, d, J=8.0 Hz), 7.76 (2H, d, J=8.4 Hz), 8.56 (1H, s), 9.69 (1H, br s). *Anal.* Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.68; H, 5.23; N, 14.36.

2-Amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine (2)[9)] To a solution of **6** (293 mg, 1 mmol) in MeOH–CH_2Cl_2=1 : 30 (60 ml) was added Zn powder (586 mg) and AcOH (0.18 ml, 3 mmol). After being refluxed for 20 min with vigorous stirring, the mixture was filtered and the filtrate was diluted with AcOEt. The system was then washed with H_2O and 10% $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. Silica gel column chromatography (MeOH–CH_2Cl_2=1 : 15) of the residue gave a crystalline solid which was crystallized from ether-hexane to give 2-aminopyrazine (**2**) (263 mg, 95%) as pale yellow prisms, mp 217—219 °C.[2c,4)] ^{1}H-NMR (DMSO-d_6) δ: 4.05 (2H, s), 6.19 (2H, s), 6.79 (2H, d, J=8.8 Hz), 7.18 (1H, t, J=7.7 Hz), 7.27 (2H, t, J=7.7 Hz), 7.33 (2H, d, J=8.0 Hz), 7.72 (2H, d, J=8.8 Hz), 8.28 (1H, s), 9.51 (1H, br s).[4)] *Anal.* Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.77; H, 5.51; N, 15.20.

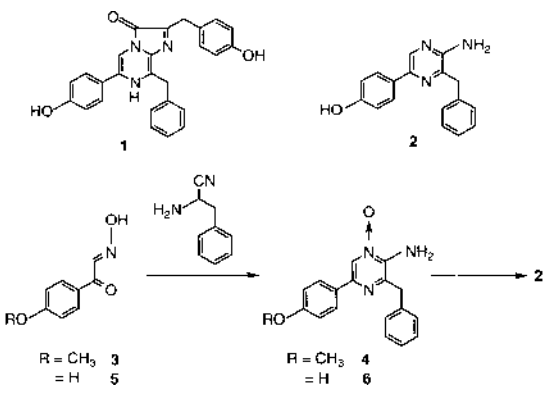

Fig. 1

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References and Notes

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9) This compound was submitted to the facile one-step cyclization reported in the previous paper.[5)] That is, a mixture of **2** and excess *p*-hydroxyphenylpyruvic acid (7 equiv.) was heated in dioxane at 130 °C under gentle refluxing. After 30 min, the solvent was then allowed to evaporate almost to dryness at 140 °C. The resultant dark resinous residue was purified by chromatographic work to give **1** in 63% yield.