IMPROVED SYNTHESIS OF WATASENIA PRELUCIFERIN

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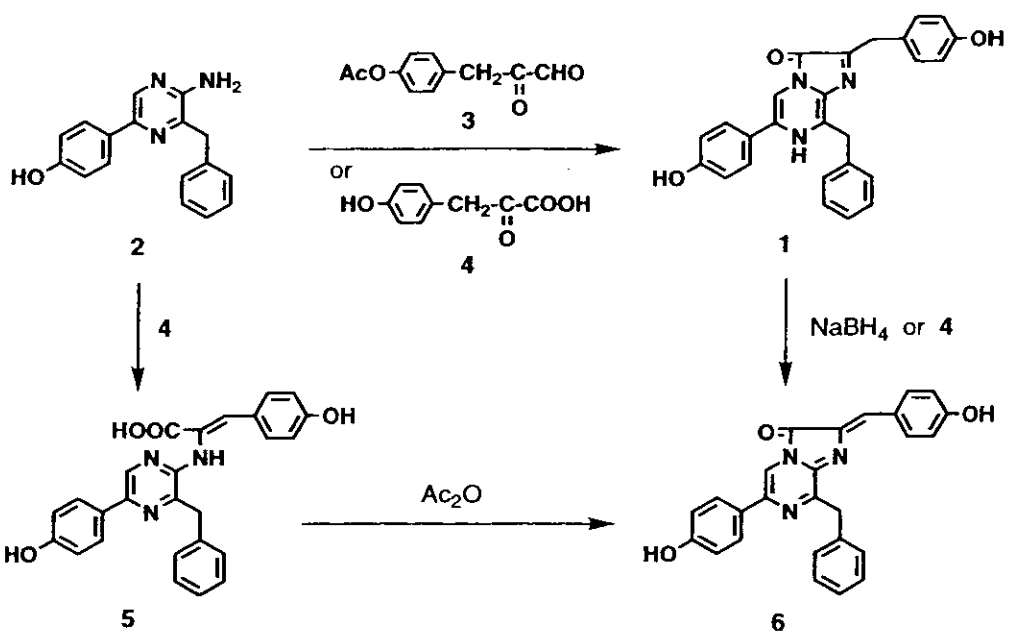
Abstract - On heating a mixture of 2-amino-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine (**2**) and a large excess of *p*-hydroxyphenylpyruvic acid (**4**) at 130-140 ℃ in dioxane, without any reductive treatment, Watasenia preluciferin (**1**) was obtained directly in one batch process in 63% yield.

Watasenia preluciferin (coelenterazine) (**1**), widely distributed in marine luminescent animals, was first isolated by the authors in 1975 from the liver of the squid, *Watasenia scintillans* (in Japanese, Hotaruika).[1] Subsequent studies on marine bioluminescent systems indicated **1** to be essential to light emitting systems of various marine luminescent organisms[2] such as squid,[3] shrimp,[4] coelenterate[5] and fish.[6] This compound was synthesized to confirm its structure[1] and was derived to various bioluminescent compounds isolated from the above animals.[3-6]

For the first synthesis of **1** in 1975, 2-aminopyrazine (**2**) was treated with *p*-acetoxybenzylglyoxal (**3**) in acidic medium to give **1** in 68% yield.[1] This method proved effective for the cyclization of imidazopyrazinone nuclei but compound (**3**) required six steps for its preparation, starting from *p*-hydroxyphenylacetic acid.



In 1980, commercially available *p*-hydroxyphenylpyruvic acid (**4**) in place of **3** was noted to react with **2** in *t*-BuOH-dioxane under reflux conditions, with no need for reductive treatment, to obtain **1** in 49% yield along with dehydropreluciferin (**6**) at 8.4%.[7] In this reaction, on heating a mixture of **2** and **4** (2 equiv) in dioxane at 130 ℃ for one hour, unstable dehydroamino acid (**5**)[8] was obtained. The cyclization of **5** using Ac_2O in dioxane gave dehydropreluciferin (**6**)[9] which was readily reduced with sodium borohydride to give Watasenia preluciferin (**1**) in good yield. It was considered that the terminative product between **2** and **4** under ordinary reaction conditions, without reductive treatment,[10] would be dehydropreluciferin (**6**), but actually, only Watasenia preluciferin (**1**) was obtained in 63% yield by merely heating **2** in excess **4** (7 equiv) in dioxane at 130-140 ℃. Treatment of **6**, in place of **2**, with **4** (7 equiv) in dioxane under the same conditions as for **2** also gave **1** in 40% yield. Accordingly, excess pyruvic acid in the above reactions would thus appear to function as a hydrogenating agent for **6**. Compound (**1**) and related bioluminescent derivatives[3-6] are difficult to obtain from natural sources and thus the cyclization of 2-aminopyrazine (**2**) with commercially available *p*-hydroxyphenylpyruvic acid (**4**) leading to **1** directly in one batch process is quite useful for the synthesis of **1** and related compounds.

Experimental

The experiments were carried out with microscale glassware apparatus. Melting points were uncorrected. Spectra were recorded by the following instruments; IR spectra, JASCO IRA-1 spectrophotometer; UV spectra, Hitachi 323 spectrophotometer; MS spectra, Hitachi M-80B spectrometer; NMR spectra JEOL JNM FX-100 or A-600 spectrometer. Chemical shifts of NMR spectra are given in ppm from tetramethylsilane as the internal standard. HPLC separations were carried out on JASCO Triroter V. Preparative TLC was conducted on Merck Kieselgel $60F_{254}$ and column chromatography was performed on Merck Kieselgel 60 (70-230 mesh) .

Watasenia dehydroamino acid (5)

A mixture of 2-amino-3-benzyl-5-*p*-hydroxyphenylpyrazine (**2**)[11] (14 mg, 0.05 mmol) and *p*-hydroxyphenylpyruvic acid (**4**) (18 mg, 0.1 mmol) in dioxane (0.2 mL) was heated at 130 ℃ with stirring. After 30 min, the mixture was evaporated to dryness in vacuo and the residue was purified by silica gel TLC (MeOH-CH_2Cl_2=1 : 7) to give two fractions:

Fraction 1: recovered **2** was washed with a small amount of ether to afford a pale yellow crystalline solid (1 mg) . This fraction contained a trace amount of dehydropreluciferin (**6**) which was characterized by TLC (acetone- CH_2Cl_2=1 : 10).[9]

Fraction 2: an unstable pale yellow crystalline solid of dehydroamino acid (**5**) (18.2 mg, 82%); mp gradually decomposed over the range of 130-160 ℃; ^{1}H NMR (DMSO-d_6) 4.25 (2H, s), 6.44 (2H, d, *J*=8 Hz), 6.73 (2H, d, *J*=8 Hz), 7.0-7.4 (8H, m), 7.64 (2H, d, *J*=8 Hz), 7.74 (1H, br s), 8.14 (lH, s). This compound was converted to the stable methyl ester by treatment with diazomethane (2% diazomethane in ether, rt, 30 min). The methyl ester of **5**: pale yellow prisms (from MeOH); mp 208-210 ℃ (decomp); MS *m/z* 453 (M^+); UV (MeOH) λ_{max} 287 (log ε 4.52), 355sh (4.30) nm; UV (MeOH-

NaOH) λ_{max} 307 (log ε 4.47), 368 (4.43) nm; IR (KBr) 1722, 1606, 1583, 1482, 1368, 1245 cm^{-1}; ^1H NMR (DMSO-d_6) 3.60 (3H, s), 4.28 (2H, s), 6.53 (2H, d, *J*=8 Hz), 6.73 (2H, d, *J*=8 Hz), 7.0-7.5 (8H, m), 7.71 (2H, d, *J*=8 Hz), 8.09 (1H, br s), 8.25 (1H, s), 9.46 (1H, br s), 9.71 (1H, br s); *Anal.* Calcd for $C_{27}H_{23}N_3O_4$: C, 71.51; H, 5.11; N, 9.27%, Found: C, 71.47; H, 4.91; N, 8.80%.

Watasenia dehydroprelucifelin (6)

A mixture of **5** (30 mg, 0.068 mmol) and Ac_2O (7 μL, 0.068 mmol) in dioxane (0.3 mL) was allowed to stand at rt for 30 min. The resultant dark colored reaction mixture was evaporated to dryness in vacuo and the residue was purified by silica gel TLC (MeOH-CH_2C1_2=1 : 10) to give a dark colored crystalline solid (26.6 mg, 92.4%); mp 250 ℃ (decomp); MS *m/z* 421 (M^+) ; HPLC 24 min (column; Nomura Chemical Co., Develosil Packed column ODS-5, 10×250 mm, solvent; 90% MeOH-H_2O, flow rate 1 mL/min) ; UV (EtOH) λ_{max} 274 (log ε 4.35), 425 (4.39), 536 (4.13), 574 (4.10) nm; UV (EtOH-NaOH) λ_{max} 295 (log ε 3.89), 546 (4.41), 587 (4.37), 629 (4.25) nm; ^1H NMR (DMSO-d_6) 4.31 (2H, s), 6.76 (2H, d, *J*=8 Hz), 6.93 (2H, d, *J*=8 Hz), 7.1-7.6 (6H, m), 7.75 (2H, d, *J*=8 Hz), 7.87 (1H, s), 8.28 (2H, d, *J*=8 Hz), 9.56 (1H, br s). Physical properties of this product were identical with those of compound (**6**) prepared from **1** by oxidation with MnO_2 in ether-EtOH under ice-cooling followed by the usual work up.[9]

Reaction of 2-aminopyrazine (2) and *p*-hydroxyphenylpyruvic acid (4)

A mixture of **2** (55.4 mg, 0.2 mmol) and **4** (252 mg, 1.4 mmol) in dioxane (1 mL) was heated at 130 ℃ under gentle refluxing. After 30 min, the temperature was rised to 140 ℃ and the solvent was allowed to evaporate almost to dryness. After being heated for an additional 15 min at 140 ℃, the dark resinous residue[12] was taken up in MeOH-CH_2Cl_2 (1 : 5) and subjected to silica gel column chromatography (MeOH- CH_2Cl_2=1 : 5) followed by silica gel TLC (MeOH- CH_2Cl_2=1 : 7) to give an orange yellow crystalline solid (53.4 mg, 63.1%); orange yellow prisms (from MeOH), mp 175-178 ℃ (decomp); MS *m/z* 423 (M^+); HPLC 13.0 min (column; Nomura Chemical Co., Develosil Packed column ODS-5, 10×250 mm, solvent; 90% MeOH-H_2O, flow rate 1 mL/min). This compound was identical with Watasenia preluciferin (**1**) in all respects.[1] IR (KBr) 3650, 3250-3070br, 1670, 1620, 1615, 1560, 1515, 1455, 1240, 1160 cm^{-1}; UV (MeOH) λ_{max} 263 (log ε 4.38), 430 (3.98) nm; UV (MeOH-HCl) λ_{max} 282 (log ε 4.41), 356 (3.85) nm; UV (MeOH-NaOH) λ_{max} 286 (log ε 4.39), 403 (3.88) nm; ^1H NMR (600 MHz, CD_3OD) 4.07 (2H, s), 4.40 (2H, s), 6.69 (2H, d, *J*=8 Hz), 6.88 (2H, d, *J*=8 Hz), 7.15 (2H, d, *J*=8 Hz), 7.23 (1H, t, *J*=7 Hz), 7.29 (2H, t, *J*=7 Hz), 7.45 (2H, br s).

Reaction of Watasenia dehydropreluciferin (6) and *p*-hydroxyphenylpyruvic acid (4)

A mixture of **6** (10 mg, 0.024 mmol) and **4** (30 mg, 0.167 mmol) in dioxane (0.2 mL) was treated under the same conditions as for **2** and **4** described above. The resulting product (4.0 mg, 40%) was found identical with **1**.

Reduction of 6 with sodium borohydride

$NaBH_4$ (5mg, 0.217 mmol) was added portion wise to an ice-cooled solution of **6** (10 mg, 0.024 mmol) in MeOH-dioxane (1:1, 1 mL) with stirring. On completion of the reduction (about 10 min as being judged by TLC), the mixture was evaporated to dryness in vacuo and the residue was taken up in MeOH-CH_2Cl_2 (1 : 3) and subjected to silica gel TLC (MeOH- CH_2Cl_2=1 : 7) to give **1** (10 mg, 99.5%) as an orange yellow crystalline solid.

REFERENCES AND NOTES

1. S. Inoue, S. Sugiura, H. Kakoi, K. Hashizume, T. Goto, and H. Iio, *Chem. Lett.*, **1975**, 141.
2. O. Shimomura, S. Inoue, F. H. Johnson, and Y. Haneda, *Comp. Biochem. Physiol.*, 1980, **65B**, 435.
3. S. Inoue, H. Kakoi, K. Okada, H. Tanino, and T. Goto, *Agric. Biol. Chem.*, 1983, **47**, 635.
4. S. Inoue, H. Kakoi, and T. Goto, *Chem. Comm.*, **1976**, 1056.
5. S. Inoue, H. Kakoi, M. Murata, T. Goto, and O. Shimomura, *Chem. Lett.*, **1979**, 249.
6. S. Inoue, K. Okada, H. Tanino, and H. Kakoi, *Chem. Lett.*, **1987**, 417.
7. S. Inoue, K. Okada, H. Tanino, and H. Kakoi, *Chem. Lett.*, **1980**, 299.
8. Assignment of *E*, *Z* stereochemistry of the double bond remains unknown.
9. S. Inoue, H. Taguchi, M. Murata, H. Kakoi, and T. Goto, *Chem. Lett.*, **1977**, 259.
10. For the first synthesis of Cypridina luciferin (in Japanese, Umihotaru luciferin), reduction was required when 2-amino-3-(γ-guanidinopropyl)-5-(β-indolyl)pyrazine was treated with aqueous solution of 3-methyl-2-oxovaleric acid. Y. Kishi, T. Goto, S. Inoue, S. Sugiura, and H. Kishimoto, *Tetrahedron Lett.*, **1966**, 3445.
11. Y. Kishi, H. Tanino, and T. Goto, *Tetrahedron Lett.*, **1972**, 2747.
12. No dehydropreluciferin (**6**) by TLC monitoring (MeOH- CH_2Cl_2=1 : 10) .

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