SYNTHESIS OF CYTOTOXIC PYRROLOQUINAZOLINOQUINOLINE ALKALOID LUOTONIN A

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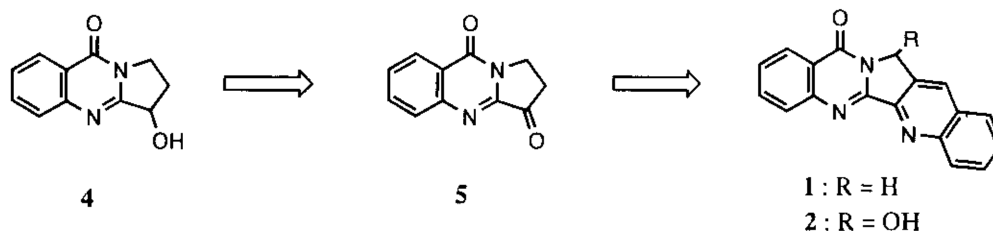
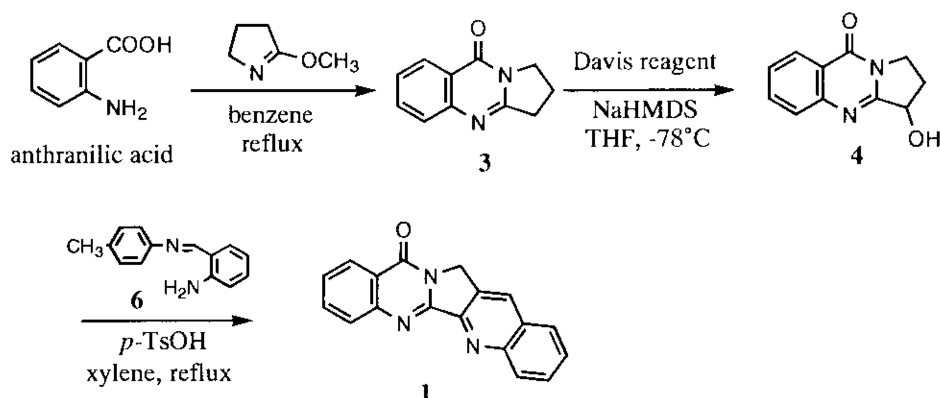
Abstract - A convenient synthesis of cytotoxic pyrroloquinazolinoquinoline alkaloid, luotonin A (**1**), was achieved in 3 steps; (i) reaction of anthranilic acid with *O*-methylbutyrolactim to deoxyvasicinone, (ii) oxidation of deoxyvasicinone, and (iii) formation of luotonin A by the reaction of vasicinone with anthranyl aldehyde.

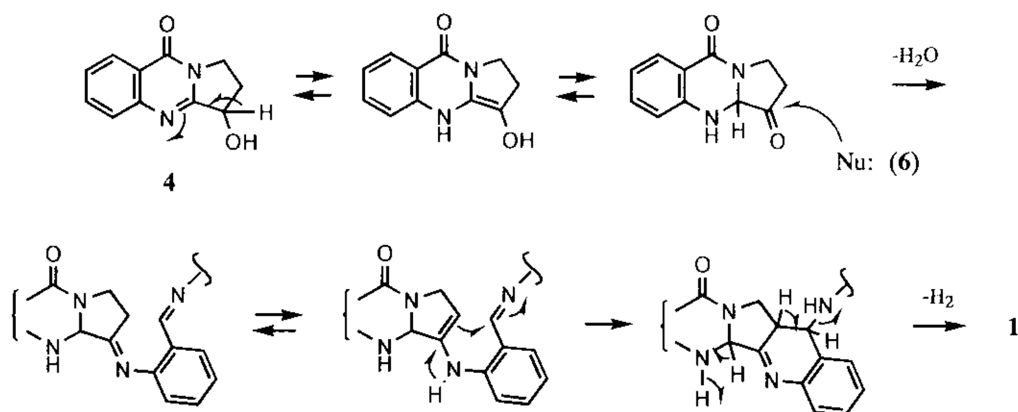
Luotonins A (**1**) and B (**2**) are novel pyrroloquinazolinoquinoline alkaloids isolated from *Peganum nigellastrum* and **1** showed cytotoxic activity against mouse leukemia P-388 cells (IC_{50} 1.8 μg/mL).[1] The compound (**1**) is attractive compound with respect to anti-tumor activity, because of its structural similarity to camptothecin,[2] an inhibitor of DNA topoisomerase type-I. From this point of view, we examined the synthesis of luotonin A (**1**). Recently, Ganesan *et al.* achieved the total synthesis of **1** by the coupling reaction of 3-oxo-pyrroloquinoline with 2-sulfinylaminobenzoyl chloride in the presence of $LiN(TMS)_2$.[3] On the other hand, main pyrroloquinazoline alkaloids vasicine, deoxyvasicinone (**3**), and vasicinone (**4**) have been also isolated from the aerial parts of *P. nigellastrum.*[1] Luotonin A (**1**) may be derived from the pyrroloquinazoline alkaloids, such as vasicinone (**4**), through a plausible biosynthetic pathway as described in Scheme 1.[1] Pyrroloquinazoline alkaloids, including **3** and **4**, in *P. harmala* have been found to be biosynthesized through condensation of anthranilic acid with ornithine- or glutamic acid-derived amino acids.[4] Actually condensation reaction of anthranilic acid derivative with 2-pyrrolidone-5-carboxylic acid,[5] 2-pyrrolidone[6] and *O*-methylbutyrolactim[7] has been applied to synthesize the pyrroloquinazoline skeleton. This paper descibes the total synthesis of **1** according to the biogenetic route involving a quinazoline alkaloid as a key intermediate.



Scheme 1 Biogenetic route to luotonins A (**1**) and B (**2**) from vasicinone (**4**)



Scheme 2 Synthetic route to luotonin A (**1**) from anthranilic acid



Scheme 3 One of tautomers of **4** acts as reaction species in the formation of luotonin A (**1**)

Firstly, deoxyvasicinone (**3**), one of putative precursors of **1**, was prepared from anthranilic acid and *O*-methylbutyrolactim[8] in 93 % yield by Onaka's procedure[7a,9] (Scheme 2). Subsequently, the oxidation reaction of **3** employing (1*S*)-10-camphorsulfonyloxaziridine, *i. e.*, the Davis reagent by Eguchi *et al.*,[10] gave vasicinone (**4**) in 55 % yield. Next, introduction of a quinoline ring system to the quinazoline skeleton was examined according to the biogenetic route depicted in Scheme 1, in which pyrrolo[2,1-*b*]quinazoline-3,9-dione (**5**) was regarded as a significant precursor leading to **1**. However, oxidative reaction of **4** by the conventinal ways did not give the expected product (**5**). Meanwhile, when a mixture of vasicinone (**4**), *N*-(2-aminobenzylidene)-*p*-toluidine[11] (**6**) and *p*-toluenesulfonic acid in dry xylene was heated under reflux for 20 h, the reaction proceeded smoothly to give luotonin A (**1**) in 30 % yield. The physical and NMR data of the resulting **1** completely agreed with those of natural specimen. In this reaction, one of tautomers of **4**, that is a keto-form derivative, presumably acts as reaction species in the formation of **1** (Scheme 3). Furthermore, upon addition of 1.0 equimolar amount of *p*-benzoquinone to the reaction mixture, the yield of **1** was improved up to 46 %, in which *p*-benzoquinone would stimulate

dehydrogenation process. At this time, the total yield of **1** from the starting material was 23.5 %. The total synthesis of luotonin A (**1**) was thus accomplished in convenient 3 steps.

As mentioned above, pyrrolo[2,1-*b*]quinazoline-3,9-dione (**5**) was regarded as a significant precursor leading to **1** in a plausible biosynthetic route (Scheme 1). However, direct conversion of **4** into **1** by way of the keto-form intermediate suggested that vasicinone (**4**) is preferable as a pivotal precursor to **5**.

On the other hand, in the previous paper,[1] we reported the conversion of **1** to luotonin B (**2**) by a photo-oxidative reaction. Further examination for the synthesis of **2** was carried out by the reaction of **1** with an oxidizing reagent CAN. A mixture of luotonin A (**1**) and CAN in CH_3CN was refluxed for 6 h. The mixture was purified by preparative TLC to give luotonin B (**2**). In this experiment, the yield of **2** was 15 % to 5 % by the photo-reaction.

The present study provided a synthesis of the new type of pyrroloquinazolinoquinoline alkaloid luotonin A (**1**, the overall yield was 23.5 %) by convenient method.

EXPERIMENTAL

Melting points were determined by Yanaco micro-melting point apparatus MP-500V and are uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FTIR 300 spectrophotometer and UV spectra were recorded on a Shimadzu 265 UV spectrophotometer. NMR spectra were recorded on a JEOL JNM EX-400 FTNMR spectrometer. MS spectra were recorded on a JEOL JMS DX-303 spectrometer.

Deoxyvasicinone (3) 137 mg (1.0 mmol) of anthranilic acid was suspended in 15 mL of benzene at 5 °C, and 200 mg (2.0 mmol) of *O*-methylbutyrolactim was slowly added. After stirred for 2 h at the same temperature, the mixture was heated and stirred at 30 - 40 °C for 1 h, and then refluxed for another 5 h. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC [$CHCl_3$ - acetone (3 : 1)] to give 174.2 mg (93 %) of deoxyvasicinone (**3**) as a colorless needles: mp 106 - 108 °C (lit.,[6b] 106 - 108 °C); UV λ max(MeOH) nm (log ε): 206 (4.51), 224 (4.54), 265 (3.98), 302 (3.68), 313 (3.60); IR (KBr): 1678, 1614, 1559, 1469, 1425, 1385, 1336, 774, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.29 (2H, m), 3.18 (2H, t, J = 8.0 Hz), 4.21 (2H, t, J = 7.2 Hz), 7.45 (1H, dt, J = 1.3 and 7.5 Hz), 7.65 (1H, d, J = 7.5 Hz), 7.72 (1H, dt, J = 1.6 and 7.6 Hz), 8.28 (1H, dd, J = 1.6 and 8.0 Hz); EIMS: m/z (rel. int.) 186 (M^+, 100), 160 (23), 130 (40), 102 (46). The spectral data were identical with reported one[6b,7a] and our authentic sample.[1]

Vasicinone (4) In a 30 mL oven dried two-necked round bottomed flask fitted with a nitrogen bubbler, a rubber septum, and a magnetic stirring bar was placed 183 mg (1.0 mmol) of NaHMDS in 2 mL of dry THF, and cooled to -78 °C. Slow and dropwise addition *via* syringe to this solution was 93 mg (0.5 mmol) of deoxyvasicinone (**3**) in 3 mL of dry THF, and the mixture was stirred for 1 h at the same temperature followed by addition of 229 mg (1.0 mmol) of (*S*)-Davis reagent in 4 mL of dry THF. After 1.5 h, the reaction was quenched at -78 °C by addition of saturated aqueous NH_4Cl (1 mL) and warmed to rt. The mixture was diluted with AcOEt, and the combined organic layers were washed with water and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue

was purified by preparative TLC [$CHCl_3$ - acetone (2 : 1)] to give 55.5 mg (55 %) of vasicinone (**4**) as a white solide: mp 205 - 207 °C; $[\alpha]_D$ +14° (*c* = 0.20, $CHCl_3$); UV λ max(MeOH) nm (log ε): 206 (4.46), 225 (4.46), 267 (3.92), 301 (3.63), 313 (3.55); IR (KBr): 3201, 1682, 1627, 1469, 1330, 1266, 775, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.31 (1H, m), 2.67 (1H,m), 4.06 (1H, m), 4.37 (1H, m), 5.26 (1H, t, J =7.2 Hz), 7.51 (1H, dt, J = 1.5 and 8.1 Hz), 7.76 (2H, m), 8.31 (1H, ddd, J = 0.7, 1.5 and 8.1 Hz); EIMS: m/z (rel. int.) 202 (M^+, 100), 146 (70), 119 (52). The spectral data were identical with reported one[10] and our authentic sample.[1]

Luotonin A (1) A mixture of 30 mg (0.14 mmol) of vasicinone (**4**), 62 mg (0.28 mmol) of *N*-(2-aminobenzylidene)-*p*-toluidine, 16 mg (0.14 mmol) of *p*-benzoquinone, 5.5 mg of *p*-toluenesulfonic acid and 2.5 g of molecular sieves 4A 1/16 in 10 mL of dry xylene was heated to mild reflux for 20 h in a nitrogen atmosphere. After poured out the solvent, the solid was extracted with $CHCl_3$ - MeOH (3 : 1, 25 mL x 3, 10 min) by ultrasonication. The combined organic layer was removed under reduced pressure, and the residue was purified by preparative TLC [benzene - acetone (8 : 1), $CHCl_3$ - acetone (10 : 1)] to give 19.5 mg (46 %) of luotonin A (**1**) as pale yellow needles after crystallization from $CHCl_3$: mp 281 - 283 °C [natural product 281 - 283 °C ($CHCl_3$)]. The spectral data of synthetic luotonin A were completely agreement with those of our natural sample.[1]

Luotonin B (2) A mixture of 10 mg of luotonin A (**1**) and 30 mg of CAN in 10 mL of CH_3CN was heated to reflux for 6 h. After evaporated the solvent, the residue was purified by preparative TLC [$CHCl_3$ - acetone (10 : 1)] to give 1.6 mg (15 %) of luotonin B (**2**) as pale yellow crystalline powder, mp 237 - 240 °C, along with starting material (5.2 mg). The spectral data of synthetic luotonin B were agreement with those of our natural sample.[1]

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