Facile Synthesis and Radioiodine Labeling of Hypericin

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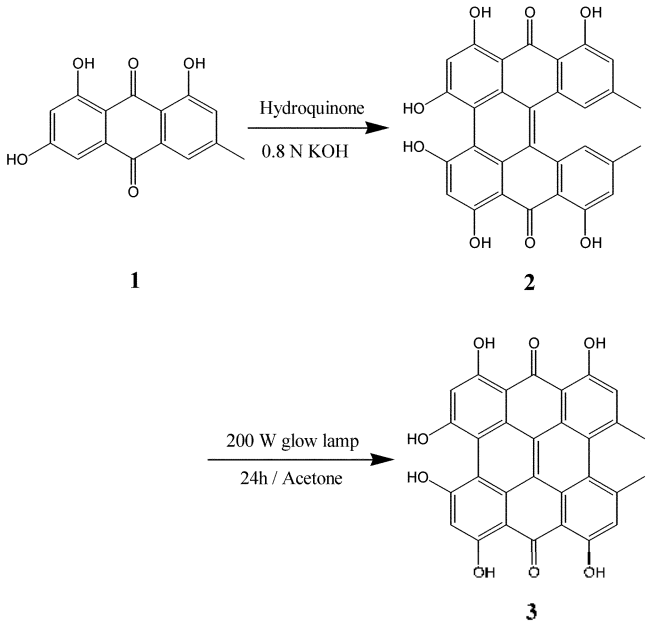
Hypericin (1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenanthro[1,10,9,8-opqra]perylene-7,14-dione), an antidepressant which is also known to be a potent protein kinase C (PKC) inhibitor was synthesized as a precursor for radioiodine labeling *via* two step reactions. Malignant glioma cells express higher PKC activity compared to untransformed glial cell. Here we report the synthesis and radioiodine labeling of hypericin as a potential brain tumor imaging radiopharmaceutical. The reference compound, 2-iodohypericin, and its radiolabelled analogues, 2-[^{123}I]iodohypericin and 2-[^{124}I]iodohypericin have been prepared by the reaction of hypericin with NaI or [^{123}I]NaI or [^{124}I]NaI. The labeling yield was 60-65% for each analogue and the optimal reaction time was 10 min. The purification and isolation of the labelled products were achieved by a reversed-phase HPLC.

Key Words : Hypericin, 2-[^{123}I]iodohypericin, 2-[^{124}I]iodohypericin, Protein kinase C (PKC) inhibitor, Malignant glioma

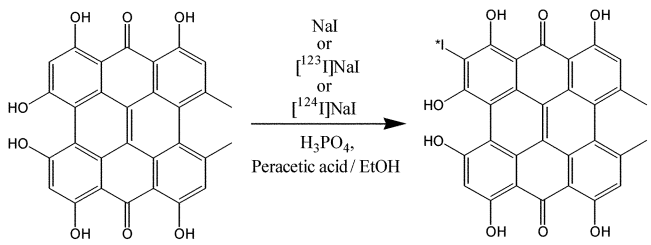
Introduction

Hypericin **3**, a natural polycyclic aromatic dianthraquinone, is mostly found in plants belonging to Hypericum genus (St. John's wort) and has been used in treatments of depression.[1] There have been intensive studies for **3** on its antiretroviral activity against several types of virus including human immunodeficiency virus (HIV)[2-5] and its potential inhibitory effect against PKC for last decade.[6] Previous studies showed that a direct correlation was found between PKC and enzyme activity in all human malignant glioma cell lines *in vitro*.[7-9] PKC is the multi functional and ubiquitous enzyme system related with signal transduction pathways. Recent interests are focused on PKC expressing level in malignant glioma cells because malignant glioma cells show elevated PKC activity compared to normal cells.[10] Other works have shown that PKC activities correlate with the proliferation rate of glioma and increased PKC activities thus reflect the malignancy of tumor cells.[11] Recent works have demonstrated that manipulations of PKC system can alter growth rates of human malignant glioma cell lines in vitro.[12,13] **3** has been demonstrated an inhibitory effect on PKC activity. In this study, a direct labeling of hypericin with I-123 or I-124 was conducted to develop a new radiopharmaceutical for the diagnosis of brain tumors having high PKC activity. A synthesis of **3** has been initially proposed by Brockmann *et al.* in 1957.[15] Although many other attempts[16] for synthesizing **3** has been carried out using either oxidative dimerization reaction or reductive coupling, procedure to separate and purify a mixture still remained troublesome and complex. Zalkow *et al.* established an



1 **2** **3**

Scheme 1. Synthetic scheme of hypericin.



3 **4** [^{123}I]**4** [^{124}I]**4**

Scheme 2. Synthesis of 2-iodohypericin.

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Table 1. Synthetic Yields of Protohypericin by Reaction Temperatures

Temp (°C)	130-145	150-160	160-175
Yield (%)	27 ± 5.4	67 ± 6.4	59 ± 3.3

efficient method for preparation of **3** (Scheme 1), where emodin **1** without reduction step to emidin anthrone was used as a starting material and directly gave **2** by reductive coupling with hydroquinone in basic solution in relatively good yield of about 50%.[1]

2-Iodohypericin **4** has been prepared by the reaction of **3** with sodium iodide (Scheme 2).

Two radioiodine nuclides, gamma ray emitting I-123 and positron emitting I-124, were used for labeling of **3**, which was achieved by either [^{123}I]NaI[17] or [^{124}I]NaI in the presence of peracetic acid with the labeling yield of 60-65%.

Results and Discussion

Although reduction yield of emodin to emodin anthrone was relatively high, dimerization of emodin anthrone was not successful in preparing **2**. We were, thus, trying to optimize the direct coupling method conducted by Zalkow *et al.* to synthesize **2**. A similar result to referenced method was observed except photocyclization. The irradiation of **2** in bright sunlight did not provide sufficient conditions for photocyclization to produce **3** while irradiation with 200 watt glow lamp gave acceptable conversion yield of about 95%. The synthetic yields of **2** were found to be affected by the reaction temperature and listed in Table 1.

The structure of **3** has been fully elucidated by ^{1}H NMR, UV-VIS spectra and mass spectrometer and was identical to reference.[16] **4** which used as a standard compound also was effectively synthesized by the reaction of **3** with sodium iodide in the presence of peracetic acid. Addition of 0.5 mL of peracetic acid and 10 min reaction time at room temperature were optimal reaction condition for iodination of **3** and the results are summarized in Table 2.

^{123}I and ^{124}I were produced by ^{124}Te(p, xn)^{124,123}I nuclear reactions with the energy of 27 → 22 MeV for ^{123}I and 17 → 8 MeV for ^{124}I.[18] The radioisotope ^{123}I ($T_{1/2}$=13.2 h, electron capture) is the gamma ray emitter and very useful radioisotopes used in single photon emission computerized tomography (SPECT). The radioisotope ^{124}I ($T_{1/2}$=4.18 d, electron capture, γ, β^+) is the only long-lived β^+ emitting radioisotope of I and can be used as a diagnostic radiopharmaceutical which label certain molecule for positron emission tomography (PET). Radioiodination was achieved by similar method to that of **4**. Multi-iodinated **3** was observed during liquid chromatograph/mass spectrometer analysis. The spectra of iodination are shown in Figure 1.

3, **4** and di-iodinated **3** were observed in Figure 1. 503 m/z indicates **3**, 629 m/z indicates 2-iodohypericin (**4**) and 755 m/z indicates di-iodinated **3**. 10 minutes reaction at room temperature was enough for iodination and longer reaction gave multi-iodinated **3**. The referenced solvent system[17] was quite suitable for the purification for which preparative HPLC was used.

Table 2. The Percent Yield of 2-Iodohypericin by Reaction Conditions

0.5 mL of Peracetic acid used			
Reaction Time (min)	10	30	60
Yield by HPLC (%)	54 ± 5.2	38 ± 7.8	15 ± 3.5
10 min reaction			
Peracetic acid used (mL)	0.5	1.0	2.0
Yield by HPLC (%)	54 ± 5.2	13 ± 6.2	5 ± 2.2

*These data were produced immediately after iodination with crude reaction mixture before purification to optimize reaction condition.

As shown in Figure 2, **3**, 2-[^{123}I]iodohypericin **[^{123}I]4** and one unknown radioactive by-product which is presumably di-radioiodinated hypericin, show well separated chromatogram. Same results have been obtained in case of labeling of hypericin with [^{124}I]NaI. Although further studies on malignant glioma cell line using **[^{123}I]4** and animal study using **[^{124}I]4** to get a PET image in mouse with glioma are ongoing, improvement of low solubility problem of **3** in water should be addressed.

Conclusion

Hypericin **3** has been prepared as a precursor for radioiodination in relatively good yield of 57%. To evaluate biological properties of **3** on malignant glioma, **[^{123}I]4** and **[^{124}I]4** have been synthesized with radiochemical yield of 65% for both. The reference compound, **4**, was elucidated by ^{1}H NMR and mass spectrometer. We have found that the optimal conditions of radioiodination of **3** were 10 min at room temperature. The biological evaluation of **[^{123}I]4** and **[^{124}I]4** is ongoing and low water solubility has to be solved for developing radioiodine labelled hypericin as a new radiopharmaceutical.

Experimental Section

Emodin, hydroquinone, peracetic acid, sodium iodide, phosphoric acid and potassium hydroxide were purchased from Aldrich Co.. All solvents were of analytical grade and used without further purification. Glass pressure tube was purchased from Ace glass. Thin layer chromatography was performed using silica gel 60 F_{254}. Melting points were measured on a MEL-TEMP device. ^{1}H NMR spectra were measured on a Varian Gemini-200 spectrometer and referenced to tetramethylsilane. Mass spectrometer was obtained with QUATTRO LC Triple Quadrupole Tandem Mass Spectrometer (EI) and ZQ 2000 (ESI). All reactions were carried out under a positive pressure of argon gas. I-123 and I-124 were produced on a MC-50 cyclotron by irradiation of $^{124}TeO_2$ enriched target at Korea Institute of Radiological and Medical Sciences (KIRAMS). The purification was achieved by HPLC (X-terra radial R Prep RP 18, 300 × 7.8

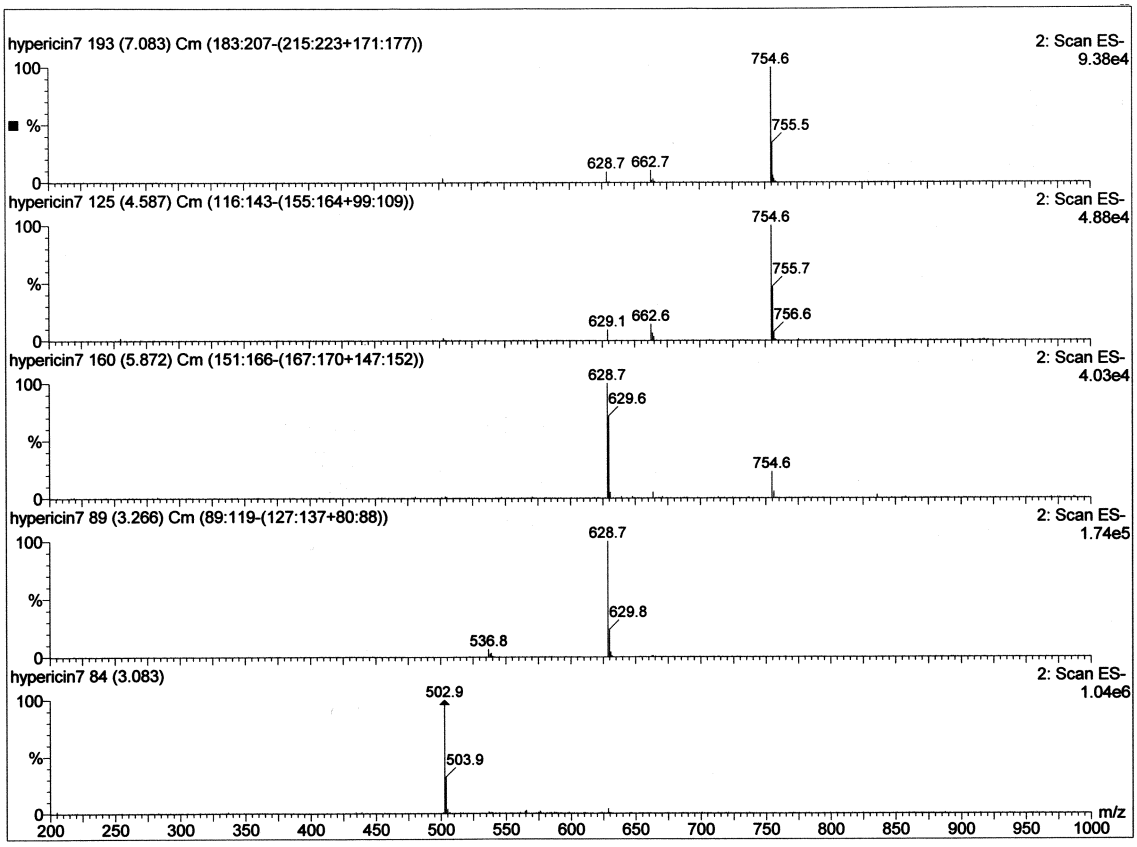

Figure 1. LC/Mass spectra of iodination of hypericin (ESI, negative). 629 m/z indicates mono-iodinated hypericin (2-iodohypericin) and 755 m/z indicates di-iodinated hypericin.

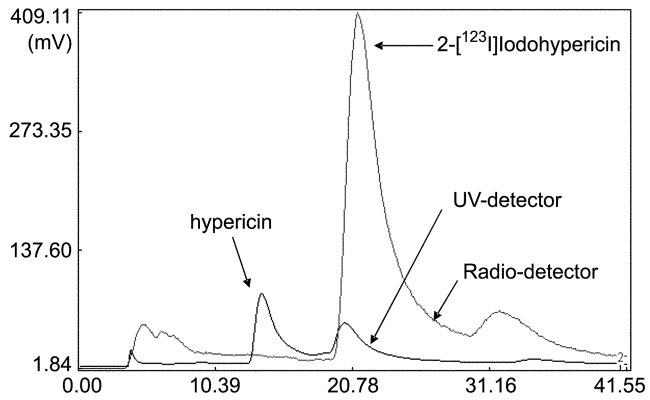

Figure 2. HPLC chromatogram of hypericin, 2-iodohypericin and 2-[^{123}I]iodohypericin. UV-Vis and radio activity chromatogram were recorded simultaneously.

mm, 10 mm, Waters; Eluent : NH_4OAc (50 mM)/EtOH, 3/7; Flow rate: 2 mL/min) with a Young-Lin M930 pump and a M720 to detect UV absorbance and a Raytest GABI series to measure radioactivity.

Protohypericin (2)[1]. 250 mg (0.93 mmol) of **1** and 210 mg (1.91 mmol) of hydroquinone were dissolved in 4.5 mL of potassium hydroxide (0.8 M). The solution contained in a glass pressure tube was flushed with argon gas to remove oxygen for more than 20 minutes and capped with teflon stopper. The solution was stirred for 1 week maintaining the temperature between 150-160 °C. The reaction mixture was cool down to room temperature and pH was adjusted to 1 with hydrochloric acid. The resulting black colored solid was filtered and washed with 200 mL of hydrochloric acid (0.1 M) and 300 mL of distilled water. Column chromatography gave 170 mg (72%) of **2**. m.p. 345-370 °C.

TLC (chloroform : methanol, 3 : 1) R_f = 0.3. ^{1}H NMR (acetone-d_6) 14.48 (s, 1H), 13.07 (s, 1H), 7.24 (s, 2H), 6.70 (s, 2H), 6.35 (s, 2H), 2.17 (s, 6H).

Hypericin (3)[1,16]. 170 mg (0.33 mmol) of **2** was dissolved in 500 mL of acetone and allowed to be irradiated with 200 watt of glow lamp for 24 hours. The color of solution has been changed from violet to dark red during irradiation. The reaction was monitored by TLC. The resulting dark red solution was evaporated and purification by column chromatograph gave 150 mg (91%) of **3**. m.p. 358-397 °C.

TLC (chloroform : methanol, 3 : 1) R_f = 0.25. ^{1}H NMR (acetone-d_6) 14.76 (s, 1H), 14.14 (s, 1H), 7.16 (s, 2H), 6.48 (s, 2H), 2.60 (s, 6H). MS (EI) M^- m/z = 504.

2-Iodohypericin (4). 180 mg (0.36 mmol) of **3** was dissolved in 60 mL of ethanol. 0.5 mL of sodium hydroxide solution (0.01 M) containing 20 mg of sodium iodide was added to ethanol solution, followed by 0.5 mL of phosphoric acid (0.5 M) and 0.5 mL of peracetic acid (32%) was added to the reaction mixture. The resulting reaction mixture was stirred for 10 minutes at room temperature. 1 mL of sodium hydrogen sulfite (0.048 M) was added to reaction mixture,

followed by 2 mL of sodium hydrogen carbonate (0.06 M) to quench the reaction and let the mixture be stirred for additional 10 minutes. Concentration *in vacuo*, column chromatography of the residue gave 63 mg (28%) of **4**. m.p. 270-310 °C.

TLC (chloroform : methanol, 3 : 1) R_f = 0.53. ^{1}H NMR (acetone-d_6) 14.76 (s, 1H), 14.14 (s, 1H), 7.16 (s, 2H), 6.48 (s, 1H), 2.60 (s, 6H). MS (ESI, negative) $[M-H]^{-1}$ m/z = 629.

2-[^{123}I]Iodohypericin ([^{123}I]4)[17]. To a reaction vial containing 150 μL of **3** solution in ethanol (30 mg/10 mL), [^{123}I]NaI solution (1.1 GBq/100 μL) was added, followed by 25 μL of phosphoric acid (0.5 M), 50 μL of 32% peracetic acid and 100 μL of ethanol were added and let the reaction mixture be stirred for 10 minutes at room temperature. The reaction was quenched by adding 50 μL of sodium hydrogen sulfite (0.048 M) and 100 μL of sodium hydrogen carbonate (0.06 M) and stirring for additional 10 minutes. The labeling reaction was monitored by radio-TLC. The labeling yield of **[^{123}I]4** was 60-65%.

TLC (chloroform : methanol, 3 : 1) R_f = 0.53.

2-[^{124}I]Iodohypericin ([^{124}I]4). 2-[^{124}I]Iodohypericin has been prepared and isolated similarly to those of **[^{123}I]4**. The labeling yield of **[^{123}I]4** was 60-65%.

TLC (chloroform : methanol, 3 : 1) R_f = 0.53.

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