Synthesis and Evaluation of 2-[^{123}I]iodoemodin for a Potential Breast Cancer Imaging Agent

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Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) is a natural chemotherapeutic compound with diverse biological properties including an antitumor activity. Emodin, a specific inhibitor of the protein tyrosine kinase, has a number of cellular targets in related to it. Its inhibition activity affects the mammalian cell cycle regulation in specific oncogene. Practically, it has been proven to inhibit *HER-2/neu* tyrosine kinase expressing breast cancer cells as an anticancer agent. 2-[^{123}I]iodoemodin has been synthesized and evaluated human breast cancer cells (MDA-MB-231, MCF-7, fibroblast as a control) which express basal levels of *HER-2/neu* tyrosine kinase to investigate its suitability as a breast cancer imaging agent and 2-iodoemodin has been synthesized as a standard compound. The radiochemical yield of the 2-[^{123}I]iodoemodin was about 72% and its radiochemical purity was over 97% after purification. The radioactivity of the 2-[^{123}I]iodoemodin was increased in a time dependent manner in both cell lines and the ratio of MDA-MB-231 and MCF7 to fibroblast was 2.9 and 1.7, respectively.

Key Words : Emodin, 2-[^{123}I]iodoemodin, *HER-2/neu*, Breast cancer, Tumor imaging

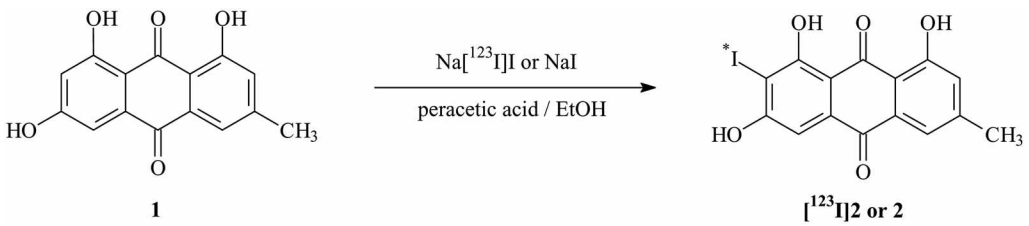
Introduction

Emodin (3-methyl-1,6,8-trihydroxyanthraquinone), a traditional herb medicine, is a natural anthraquinone and an active constituent occurring in *Rheum palmatum*.[1,2] It is believed that the hydroxyl groups in position 1 and 8 of an anthraquinone ring effect the biological action of a compound.[3] Recent pharmacological studies have revealed a number of the biological activities of emodin including an anticancer, antibacterial, anti-inflammatory effect.[4-6] It exerts an antiproliferative effect in many cancer cell lines.[7] There have been a few speculated mechanisms to explain these bioactivities such as an inhibition of cell growth,[8,9] a disruption of cell cycle,[10,11] and an induction of an apotosis,[12-14] although the mechanisms of emodin are still not clear.

Recent study showed that emodin could suppress the protein tyrosine kinase encoded from *HER-2/neu* proto-oncogene which is frequently observed in patients with breast cancer.[15,16] Tyrosine protein kinases (PTKs) play important roles in signal conduction pathways that control celluar functions such as proliferation and differentiation resulting from a tyrosine phosphorylation.[17] A number of proto-oncogenes such as *HER-2/neu* are related to PTKs which are frequently observed in tumors.[18] Particularly, uncontrolled expression of the *HER-2/neu* proto-oncogene happens in as many as 30% of breast cancer patients.[19] Emodin has been demonstrated to be a tyrosine kinase inhibitor that can suppress the activity of *HER-2/neu* proto-oncogene by blocking tyrosine phosphorylation during a signaling between ATP of the second messenger and a protein in the tumor cells.[20-22]

Thus, we postulated that if a limited structural modification of emodin, like an introduction of iodine, exerts a negligible effect on its inhibition of tyrosine, then radioiodine labeled emodin also has a possibility to visualize breast cancer.

In order to investigate radioiodine labeled emodin as a potent breast cancer imaging agent, 2-[^{123}I]iodoemodin has been prepared by the reaction of emodin with sodium [^{123}I]iodide (Figure 1). The cell uptake of 2-[^{123}I]iodoemodin



1 **[^{123}I]2 or 2**

Figure 1. Synthesis of 2-[^{123}I]iodoemodin and 2-iodoemodin.

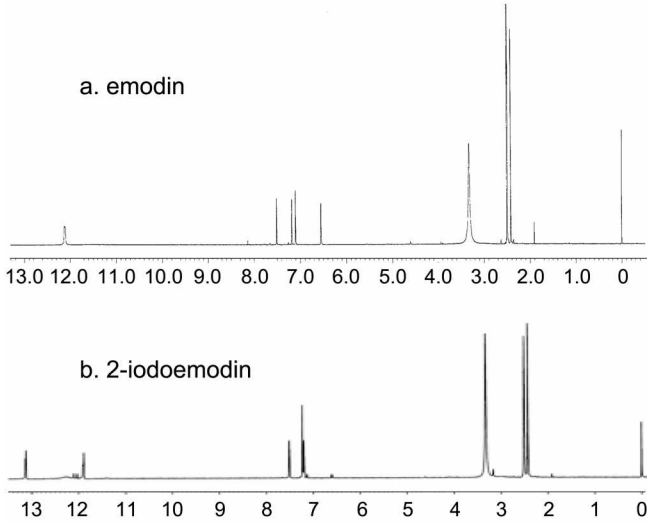


Figure 2. ^{1}H NMR spectra of emodin and 2-iodoemodin.

on human breast cancer cells (MDA-MB-231 and MCF-7) which express *HER-2/neu* tyrosine kinase was carried out.

Results and Discussion

Both the 2-iodoemodin (**2**) and 2-[^{123}I]iodoemodin ([^{123}**I**]**2**) were prepared by the reaction of emodin with sodium iodide and sodium [^{123}I]iodide in the presence of peracetic acid (Figure 1). **2** was obtained by a column chromatography of the residue (chloroform:methanol. 5:1, R_f = 0.76). The structure of **2** was confirmed by ^{1}H NMR spectroscopy and a mass spectrometry. Iodine was found to be introduced at the 2-position of the emodin from the disappearance of the proton signal of the ^{1}H NMR spectrum at 6.6 ppm which represented the H-2 signal (Figure 2).

In addition to the ^{1}H NMR data, the molecular ion peak of 396 m/z also verified the introduction of iodine to the emodin in the mass spectrum. The synthetic yield of [^{123}**I**]**2** was susceptible to the volume of the peracetic acid and the reaction temperature that is tabulated in Table 1.

As shown in Table 1, the optimal condition was 50 μL of peracetic acid, a 10 min reaction time and a room temperature reaction, whereas heating the reaction mixture (65 °C) leaded to a by-product and decreased in labeling yield (Figure 3).

Purification of [^{123}**I**]**2** was carried out by a reversed-phase

Table 1. The Yield of 2-[^{123}I]iodoemodin by the Reaction Conditions

Reaction time: 10 min	Room Temperature				Heating*			
peracetic acid (μL)	25	**50**	100	200	25	50	100	200
yield (%)	56	**72**	58	52	32	43	40	35
50 μL of peracetic acid	Room Temperature				Heating*			
time (min)	5	**10**	30	60	5	10	30	60
yield (%)	56	**72**	63	64	38	35	43	28

*The temperature was 65 and heating over 65 leaded to a low labeling yield.

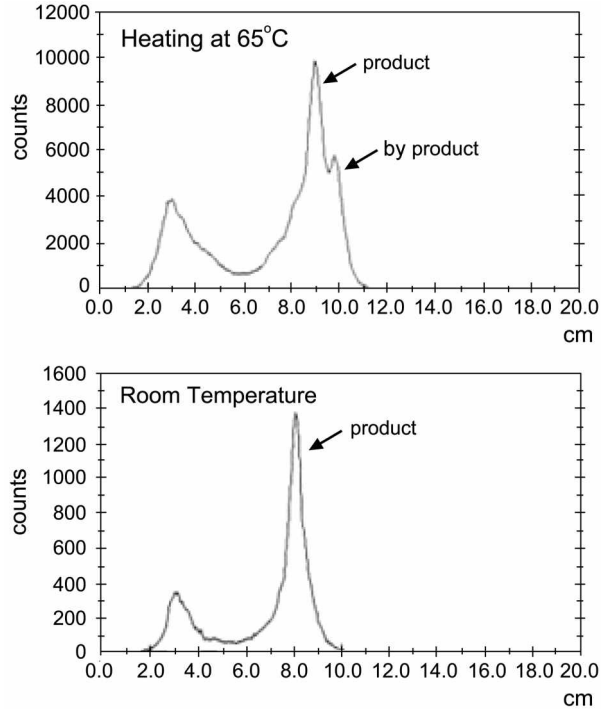


Figure 3. Radio TLC chromatogram of 2-[^{123}I]iodoemodin.

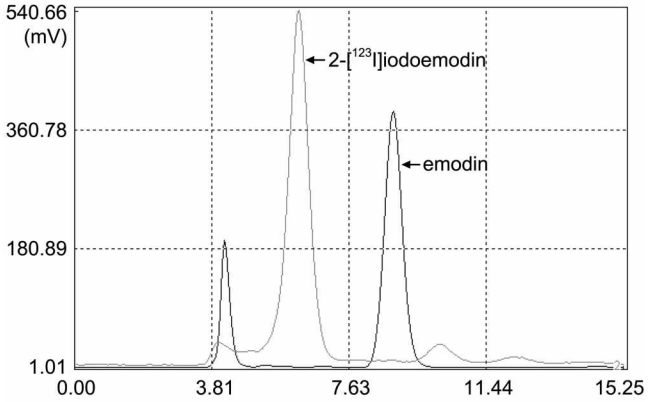


Figure 4. HPLC chromatogram of 2-[^{123}I]iodoemodin (X-Terra RP 18 prep column, 300 × 7.8 mm, 10 μm, Waters: Eluent: 0.25 M NH_4OAc/MeOH, 3/7; Flow rate: 2 mL/min).

HPLC using ammonium acetate and methanol (Figure 3). To obtain a proper preparation for the cellular uptake, the isolated solution from the semi-preparative HPLC was dried with a stream of nitrogen to remove the residual methanol.

The results of a cellular uptake of [^{123}**I**]**2** are shown in Table 2.

The cellular uptakes of [^{123}**I**]**2** increased in a time dependent manner for both breast cancer cell lines (MDA-MB-231 and MCF7) whereas a fibroblast showed a low cellular uptake of [^{123}**I**]**2**. The maximum uptake of MDA-MB-231 of [^{123}**I**]**2** was 1.7 folds higher than that of MCF-7 at 120 min. The ratio of MDA-MB-231 and MCF7 to the fibroblast was 2.9 and 1.7, respectively. These result suggests that 2-[^{123}I]iodoemodin has a potential to be used as a breast cancer imaging agent for SPECT.

Table 2. The cell uptake of 2-[^{123}I]iodoemodin for the cell lines (mean ± sd, n = 3, % ID)

	10 min	30 min	60 min	120 min
Fibroblast	0.60 ± 0.03	0.56 ± 0.02	0.58 ± 0.03	0.62 ± 0.05
MCF7	0.70 ± 0.14	0.88 ± 0.08	0.82 ± 0.15	1.05 ± 0.11
MDA-MB-231	1.13 ± 0.11	1.25 ± 0.09	1.43 ± 0.08	1.83 ± 0.12

Experiment Section

All chemicals were purchased from Sigma-Aldrich Co. All solvents were of an analytical grade and used without further purification. Thin layer chromatography and column chromatography were performed using silica gel 60 F_{254} and silica gel 60 (0.063-0.200 mm, 70-230 mesh ASTM), respectively (MERCK). Melting points were measured on a MEL-TEMP II device. ^{1}H NMR spectra was measured on a Varian Gemini-200 spectrometer and referenced to tetramethylsilane. Mass spectrum was obtained with JMS-AX505WA (JEOL). I-123 was produced on a MC-50 cyclotron (Scanditronix, Sweden) by an irradiation of $^{124}TeO_2$ enriched target at the Korea Institute of Radiological and Medical Sciences (KIRAMS). The purification was achieved by HPLC (X-Terra RP 18 prep column, 300 × 7.8 mm, 10 μm, Waters; Eluent: 0.25 M NH_4OAc/MeOH, 3/7; Flow rate: 2 mL/min) with a Young-Lin M930 pump and a M720 to detect the UV absorbance (Young-Lin) and a Raytest GABI (Raytest) series to measure the radioactivity. All cell lines were purchased from American Type Culture Collection (ATCC).

The Cell Culture. Both of the breast carcinoma MCF-7 and MDA-MB-231 cell lines were obtained from American Type Culture Collection (ATCC). Cells were grown in Dulbecco's Modified Essential Medium (Gibco) with 1.5 g/L sodium bicarbonate supplemented with 10% fetal bovine serum (FBS) and 100 mg/mL of penicillin-streptomycin (Gibco). Cells were maintained as monolayers in a humidified 5% CO_2 atmosphere, normally in T75 flasks (Falcon). The cells were trypsinated in the T75 flasks at a 80-90% confluence using (0.05% trypsin/0.02% EDTA) and were then suspended in DMEM with 10% FBS to the desired density, normally 2×10^5 cells/mL. Cell growth status and viability was monitored by an inverted phase contrast microscopy. Trypan blue exclusion was also used to assess the viability.

2-[^{123}I]iodoemodin Uptake. Both of the breast carcinoma MCF-7 and MDA-MB-231 cell lines were seeded at 5×10^5 in 6 well plates at 3 mL per well and incubated at 37 °C in a 5% carbon dioxide for 18 h for an adherence and growth. To each well in triplicate 100 uL of 2-[^{123}I]iodoemodin (370 KBq) was added. Cells were quickly washed, harvested by trypsin treatment and rewashed twice in cold phosphate buffered saline (PBS) at designated time point (10 min, 30 min, 60 min and 120 min). The cell pellets were counted by a well-type gamma counter 1480 WIZARD (WALLAC). Data was presented as a percentage of the injected radioactivity dose (%ID).

2-Iodoemodin (2). 100 mg (0.37 mmol) of emodin and 10 mL of 0.01 M sodium iodide solution were dissolved in 20 mL of ethanol, and then 2.0 mL of 0.5 M H_3PO_4 and 10.0 mL of 32% peracetic acid were added to the reaction mixture. It was stirred for 15 minutes at room temperature. After 1 mL of 0.048 M sodium hydrogen sulfite was added to the reaction mixture. 2 mL of 0.06 M sodium hydrogen carbonate was also added to quench the reaction and it was stirred for a further 5 minutes. It was poured into 50 mL of saturated NaCl solution and extracted with 50 mL of dichloromethane twice. The combined organic phases were dried over anhydrous sodium sulfate. After a filtration, solvent was removed by a rotary evaporation. Column chromatography of the residue gave 56 mg (0.14 mmol) of **2**. The yield is 38%. m.p. 198-200 °C. TLC (chloroform: methanol, 3:1) R_f = 0.76. ^{1}H NMR (DMSO-d_6) 13.22 (s, 1H), 12.89 (s, 1H), 7.46 (s, 1H), 7.18 (s, 1H), 7.12 (s, 1H), 2.42 (s, 3H). MS (EI) M^- m/z = 396.

2-[^{123}I]iodoemodin ([^{123}I]2). [^{123}I]NaI solution (2.3 GBq/100 μL) at pH 12 was added to 150 μL of emodin solution in ethanol (3 mg/mL), and then 25 μL of 0.5 M H_3PO_4, 50 μL of 32% peracetic acid and 100 μL of ethanol were successively added and stirred for 10 minutes at room temperature. The reaction was quenched by adding 50 μL of 0.048 M sodium hydrogen sulfite and 100 μL of 0.06 M sodium hydrogen carbonate and stirring for a further 10 minutes. The labeling reaction was monitored by radio-TLC. The labeling yield of [^{123}I]iodoemodin was about 70-72%. The radiochemical purity was over 95% after purification. Radio-TLC (chloroform:methanol, 3:1) R_f = 0.76.

Conclusion

In this investigation, we have synthesized and evaluated 2-[^{123}I]iodoemodin on human breast cancer cells (MDA-MB-231 and MCF-7) which express of *HER-2/neu* tyrosine kinase. The introduction of the radioactive isotope [^{123}I]-iodine to emodin is sensitive to the volume of the peracetic acid. The radioactivity of the 2-[^{123}I]iodoemodin increased in a time dependent manner and the cellular uptake of both human breast cancer cell lines were higher than that of the fibroblast. These results suggest that 2-[^{123}I]iodoemodin has a possibility as a breast cancer imaging agent for SPECT.

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