Antitumor Activity of Anthraquinone Derivatives *in vitro*

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Diels-Alder adduct of 5-hydroxy-1, 4-naphthoquinone with cyclopentadiene was easily transformed to epoxides (**3**) by the treatment of perbenzoic acid. D.-A. adduct was oxidized with potassium ferricyanide in alkaline solution and then epoxized to give anthraquinone derivative **5**. Epoxide **3** and anthraquinone **5** showed antitumor activity against P388 leukemia cells and Colon 26 cells. The values of IC_{50} of **3** and **5** were 6.97 μg/ml and 0.121 μg/ml against P388, and 5.30 μg/ml and 1.50 μg/ml against Colon 26, respectively.

The chemotherapy of neoplastic disease has become increasingly important in recent year. An indication of this importance is the establishment of a medical specialty in oncology, wherein the physician practices various protocols of adjuvant therapy. Most cancer patients now receive some form of chemotherapy, even though it is merely palliative in many cases.

Cancer chemotherapy has recieved no spectacular breakthrough of the kind that the discovery of penicilline provided for antibacterial chemotherapy. However, there has been substantial progress in many aspects of cancer research. In paticular, an increased understanding of tumor biology has led to elucidation of the mechanisms of action for antineoplastic agents. It also has provided a basis for the more rational design of new agents. Recent advances in clinical techniques, including large cooperative studies, are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds.

There are congent reasons why cancer is more difficult to cure than bacterial infections. One is that there are qualitative differences between human and bacterial cells. Bacterial cells have distinctive cell walls and their ribosomes are different from those of human cells. In contrast, the differences between normal and neoplastic human cells are merely quntitative. Another differences is that immune mechanisms and other host defences are very important in killing bacteria and other foreign cells, whereas they play a negligible role in killing cancer cells. By there very nature, the cancer cells have eluded or overcome the immune surveillance system of body. Thus, it is necessary for chemotherapeutic agents to kill every single clonogenic malignant cell, because even one can reestablish the tumor. This kind of kill is extremely difficult to effect because antineoplastic agent kill cells by first-order kinetics. A potent anticancer drug might reduce this population 10,000-fold, in which case the symptoms would be alleviated and the patient would be in a state of remission. However, the remaining hundred million leukemia cells could readily increase to the original number after cessation of therapy. Furthermore, a higher proportion of resistant cells would be present, which would mean the treatment with the same agent would achieve a lessor response than before. For this reason, multiple drug regimens are used to reduce drastically the number of neoplastic cells. Typical protocols for leukemia contain four different anticancer drugs, usually with different modes of action. The addition of immunostimulants to the therapeutic regimen helps the body's natumal defense mechanism to identify and eliminate the remaining few cancer cells.

A further complication to chemotherapy is the relative unresponsiveness of slow-growing solid tumors. Current antineoplastic agents are most effective against cells with a high growth fraction. They act to block the biosynthesis or transcription of nucleic acids or to prevent cell division by interfering with mitotic spindles. Cells in the phase of synthesis or mitosis are highly susceptible to these agents. In contrast, cells in the resting state are resisitant to many agents. Slow-growing tumors characteristically have many cells in the resting state.[1]

Toxic effects of sulfur mustard and ethyleneimine on animals were described in the nineteenth century.[2] Sulfur mustard was shown to be active against animal tumors, but it was too nonspecific for clinical use. A variety of nitrogen mustard, for example mechlorethamine, showed selective toxicity, especially to lymphoid tissue. Success in this area was followed by causious human trials that showed mechlorethamine to be useful against Hodgkin's disease and certain lymphomas.[3] Epoxides have strained ring systers as same as ethyleneimines. The use of epoxides as cross-linking agents in textile chemistry suggested that they be tried cancer chemotherapy. Simple diepoxides such as 1, 2 : 3, 4-diepoxybutane showed clinically activity against Hodgkin's disease.[4] Mytomycin C consisting with the benzoquinone and ethyleneimine groups showed anticancer activity as alkylating agents.[5] Some anthraquinone derivatives was reported as antibiotics having clinical anticancer agents.[6]

In this view points, author tried to synthesize the anthraquinone derivatives having epoxide component as a functional group with the intention of antitumor activity.

Materials and Methods

9, 10-dioxo-5-hydroxy-1, 4, 4a, 9, 9a, 10-hexahydro-1, 4-methanoanthraquinone (**1**-exo and **2**-endo) was prepared by the Diels-Alder reaction with 5-hydroxy-1, 4-naphthoquinone (348 mg : 2 mmol) and cyclopentadiene (excess) in benzene solution (5 ml) in a sealed tube at 120°C for 8h. After the evaporation of solvent and excess cyclopentadiene, the residue was submitted to silicagel column chromatography using eluant of a mixture of ethyl acetate and hexane (1 : 10). Less polar product (**1**-exo : mp 112°C) and more polar product (**2**-endo : mp 126°C) were obtained in 60 mg (12.5%) and 408 mg (85%), respectively.

9, 10-dioxo-2, 3-epoxy-5-hydroxy-1, 2, 3, 4, 4a, 9, 9a, 10-octahydro-1, 4-methanoanthraquinone (**3** : mp 152-154°C) was obtained by the treatment of **2**-endo with m-chloroperbenzoic acid in methylene chloride solution for 6h at room temperature in 65% yield.

9, 10-dioxo-5-hydroxy-1, 4, 9, 10-tetrahydro-1, 4-methanoanthra-quinone (**4** : mp 132–134°C) was obtained by the oxidation of the mixture of **1**-exo and **2**-endo with potassium ferricyanide in 5% sodium hydroxide solution in 47% yield. In this reaction, only **2**-endo was transformed to compound **4**, and **1**-exo was recovered.

9, 10-dioxo-2, 3-epoxy-5-hydroxy-1, 2, 3, 4, 9, 10-hexahydro-1, 4-methanoanthraquinone (**5** : mp 161-163°C) was prepared by the treatment of compound **4** with m-chloroperbenzoic acid in methylene chloride solution in 60% yield. All compounds were identified by spectral measurements and elemental analyses.

The P388 leukemia cells and colon 26 cells were incubated for 48h at 37°C in a medium containing n-butyric acid (4 mmol), 12-O-tetradecanoylphorbol-13-acetate (TPA) (32 mmol) and various amounts of test compounds. Smears were made from the cell suspension, Epstein-Barr virus early antigen (EBV-EA) inducing cells were stained by means of an direct immunofluorescence technique.[7] The details of the in vitro assay on EBV-EA activation have been reported.[8] in each assay, at least 500 cells were counted and the number of stained cells (positive cells) among them was recorded. Triplicate assays were preformed for each data point.

Antitumor Activity

P388 leukemia cells and Colon 26 cells were

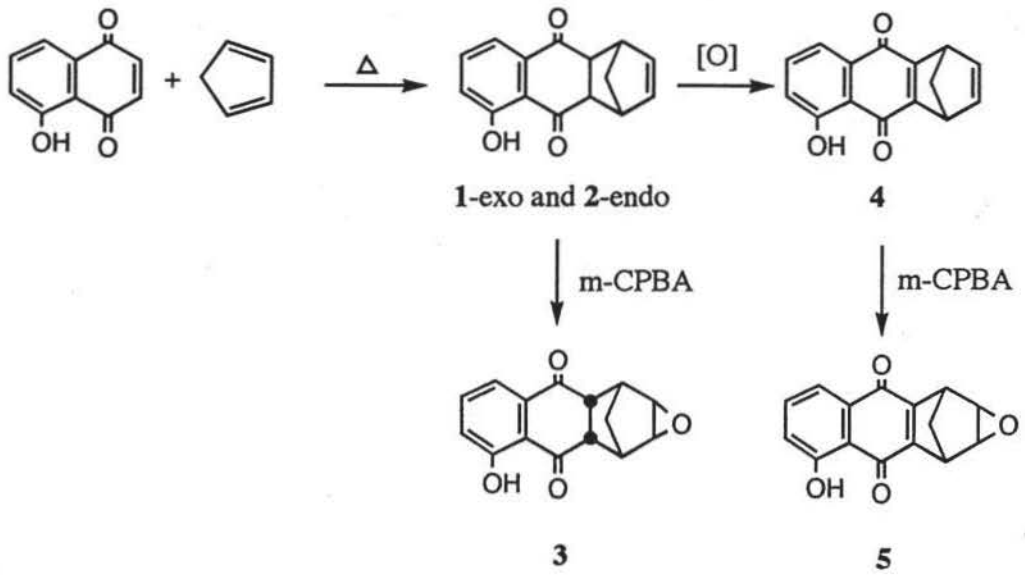

Fig. 1

Table 1 The Values of Inhibitory Concentration (ID_{50}) and Inhibitory Yields (T/C%) against P 388 and Colon 26 Cells.

	P388		Colon 26	
	ID_{50}(μg/ml)	T/C(%)	ID_{50}(μg/ml)	T/C(%)
Compound **3**	6.97	6.7	5.30	1.9
Compound **5**	0.121	10.3	1.50	0.6

used as tumor cells. At 72h in contact with P388 leukemia cells and Colon 26 cells, the maintenance medium was withdrawn, the cells were washed twice with phosphate buffered saline (PBS, pH 7.2) and fixed with acetone for 10 min at room temperature. After staining, the cells were washed with PBS and mounted with 20% glycerol in PBS. Immunofluorescence was analysed under a fluorescent microscope (Nikon Optiphot+EFD2, Nikon Industrial Co., Tokyo, Japan). When compounds **3** and **5** were contacted with these tumor cells for 72h, they showed the antitumor activity. Namely, the values of inhibitory concentration (IC_{50}) of compound **3** against p388 and Colon 26 cells were 6.97 μg/ml and 5.30 μg/ml, respectively. Compound **5** showed antitumor activity against p388 and Colon 26 cells in IC_{50} 0.121 μg/ml and 1.50 μg/ml, respectively.

Results and Discussion

The primary screening test of epoxyanthraquinone derivatives (**3** and **5**) was carried out utilizing a short-term in vitro assay on EBV-EA activation induced by a promotor, TPA. The inhibitory effect on the activation and viability of indicator cells, p388 leukemia cells and Colon 26 cells, are shown in Table 1.

These inhibitory activities were similar to those of 6-mercaptopurine (6-MP) (ID_{50} 11 μg/ml against p388; ID_{50} 1.9 μg/ml against Colon 26), which is known to be an antitumor agent.

The inhibitory mechanism of these epoxyanthraquinone derivatives on antitumor activity and their structure-activity relationships are now being studied.

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