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2-(1-Oxyalkyl)-1,4-dioxy-9,10-anthraquinones: Synthesis and Evaluation of Antitumor Activity

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Fourty eight derivatives of 2-(1-oxyalkyl)-1,4-dioxy-9,10-anthraquinone were synthesized, and their antitumor activity was evaluated. On the whole, 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinones (DHAQ=1,4-dihydroxy-9,10-anthraquinone) showed stronger cytotoxic activity againnst L1210 cells than 2-(1-hydroxyalkyl)-1,4-dimethoxy-9,10-anthraquinones(DMAQ =1,4-dimethoxy-9,10-anthraquinone), implying that free hydroxy groups at C-1 and C-4 of the anthraquinone structure are necessary for the cytotoxic activity. The bioactivity of 2-(1-hydroxyalkyl)-DHAQ derivatives differed according to the size of alkyl group at C-1;while the elongation of alkyl group over 7 carbon atoms failed to enhance the bioactivity, the derivatives possessing alkyl moiety of 1-6 carbon atoms showed an increase in the cytotoxicity and the antitumor activity in Sarcoma-180; 2-hydroxymethyl-DHAQ (ED_{50}, 15 μg/ml; T/C, 125%), 2-(1-hydroxyethyl)-DHAQ(1.9 μg/ml; 139.2%), 2-(1-hydroxypropyl)-DHAQ (7.2 μg/ml; 135.1%), 2-(1-hydroxybutyl)-DHAQ (10.2 μg/ml; 125.3%), 2-(1-hydroxypentyl)-DHAQ (23.7 μg/ml; 110.1%). and 2-(1-hydroxyhexyl)-DHAQ (58 μg/ml; 108%). Next, 2-(1-Hydroxyalkyl)-DHAQ derivatives were acetylated to produce 2-(1-acetoxyalkyl)-DHAQ analogues. Although the acetylation somewhat enhanced the cytotoxicity, but not the antitumor action. In addition, the presence of phenyl group at C-1' enhanced the cytotoxicity and the T/C value, compared to alkyl groups of same size; 2-(1-hydroxy-1-phenyl)-DHAQ (ED_{50}, 5.6 μg/ml; T/C, 137%).

Key word : 1,4-dihydroxy-9,10-anthraquinone, synthesis, S-180 and L1210 cells, structure-actitivity relationship

INTRODUCTION

Some natural anthraquinones such as alizarin, emodin and chrysophanol showed a moderate cytotoxic activity against cancer cell lines (Andreani *et al.*, 1985; Darzynkiewicz *et al.*, 1989; Itokawa *et al.*, 1993; Yoshio *et al.*, 1973; Kong *et al.*, 1992; Koyama *et al.*, 1989; Kodama *et al.*, 1987), and are often built in structure of some anticancer agents as a bioactive moiety. For instance, 1,4-dihydroxy-9,10-anthraquinone (quinizarin) is a common structural moiety of adriamycin and mitoxantrone.

The action mechanism of adriamycin is elucidated mostly as DNA intercalation (Koyama *et al.*, 1989; Abramson *et al.*, 1986; Frederick *et al.*, 1990; Islam *et al.*, 1985; Palmer *et al.*, 1988), disturbance in redox cycle system (Jeziorek *et al.*, 1993; Fisher *et al.*, 1992a; 1990b; Kuzuya *et al.*, 1991; Dodd and Mukherjee, 1984) and inhibition of DNA topoisomerase-II (Tewey *et al.*, 1984; Zunino *et al.*, 1990; Bodley *et al.*, 1989; Charcosset *et al.*, 1993). In addition, it was assumed that adriamycin undergoes bioreductive activation to produce a Michael acceptor capable of bonding covalently to cellular nucleophiles (Lin *et al.*, 1980; Patterson, 1993).

Mitoxantrone also inhibits DNA topisomerase-II (Smith *et al.*, 1990; Charcosset *et al.*, 1993; Chen *et al.*, 1986; Rosenberg *et al.*, 1986; Isabella *et al.*, 1993; Crespi *et al.*, 1986; Traganos, 1983; Krapcho *et al.*, 1991; Morier-Teissier *et al*, 1993; Kapuscinski *et al.*, 1985) and underwent, contrary to adriamycin, a biooxidative activation to give an anthradiquinone intermediate as a Michael acceptor, which covalently binds to cellular nucleophiles (Ehninger *et al.*, 1990; Mewes *et al.*, 1993; Blanz *et al.*, 1991).

On the basis of chemical model for the intercalation of adriamycin, it is supposed that its anthracyclinone moiety is positioned in the major groove of DNA and the aminosugar in minor groove (Frederick *et al.*, 1990). Therefore, it is evident that the ether bridge at C-7 between the moieties is important for its action.

The 1'-oxy group in the structure is expected to en-

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sure a dual function, a DNA intercalation and a bioreductive activation. The bioreductive activation would be dependent on properties of side chain as leaving group.

In this respect, the present study intended to synthesize 2-(1-oxyalkyl)-1,4-dioxyanthraquinone derivatives, oxy group at C-1' of side chain which correspond to 7-oxy moiety of adriamycin structure, and to evaluate the antitmor activity.

MATERIALS AND METHODS

Chemicals and solvents of reagent grade were used without further purification. L1210 cells were obtained from Korea Research Institute for Chemical Technology. RPMI 1640, fetal bovine serum and other reagents used for cell culture were purchased from Gibco Co.

Melting point was determined on an Electrothermal melting point apparatus and not corrected. IR spectra were recorded on a Jasco Report-100 IR spectrometer. Proton NMR spectra were recorded on a Varian-Gemini 200 MHz or Jeol-90 MHz spectrometer using tetramethylsilane as an internal standard.

Analytical thin layer chromatography was performed on plastic sheet (0.2 mm) precoated with silica gel 60 F254 (E. Merck). Silica gel 60 (70~230 mesh, E. Merck) was used for column chromatography.

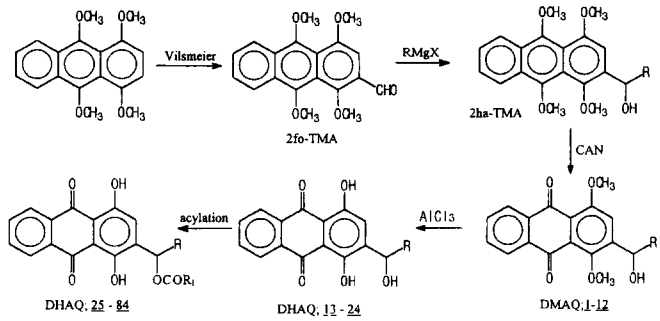
Cytotoxic activity against L1210 cells

Cytotoxicity was measured against L1210 cells *in vitro* using the known method (Thayer *et al.*, 1971). Fisher's medium supplemented with horse serum in 10% was used for the proliferation of L1210 cells. Cell numbers were counted using a haemacytometer, and ED_{50} value was defined as the concentration of drug to produce a 50% reduction in viability relative to the control in three independent experiments.

Antitumor activity in ICR mice bearing Sarcoma 180 cells

Sarcoma 180 cells suspended in saline (1×10^7 cells/ml) were inoculated intraperitoneally (injection volume: 0.1 ml per mouse) (National Cancer Institute USA, 1972). After 24 hours from the transplantation, mice were divided so that each group contains 8 mice. The test sample was dissolved in a predetermined amount of dimethylsulfoxide to prepare the stock solution which was stored at 4°C. The schedule for injection comprises a total of 7 injection in the manner that after the transplantation of cancer cells. 0.1 ml of the test sample per a day is administered continually 7 days.

The survival rate (T/C, %) was calculated by the following equation.



Scheme I. Synthesis of 2-(1-oxyalkyl)-1,4-dioxy-9,10-anthraquinone derivatives.

$$\text{Prolongation of life span (\%)} = \frac{\text{Average life span of sample treated animals}}{\text{Average life span of control animal}} \times 100$$

2-Formyl-1,4,9,10-tetramethoxyanthracene(2fo-TMA): 50 mmol (15 g) 1,4,9,10-Tetramethoxylanthracene dissolved in 80 ml dichloromthane was placed in a 500 ml two-necked flask, to which 150 mmol (10.8 g) dimethylformamide was added. The mixture was cooled down to 5°C, and 150 mmol (23 g) phosphoryl trichloride was added dropwise to the mixture for 10 min under stirring. Then, the temperature of the reaction mixture was elevated to 50°C and stirred for 4 hr. After the reaction, the mixture was added to ice water, adjusted to pH 7.0 with 1% NH_4OH, and stirred for 2 hr. The reaction mixture was extracted 3 times with 200 ml dichlormethane. After dehydration with anhydrous sodium sulfate, the dichloromethane solution was decanted and evaporated to a red brown mass, which was dissolved in dichloromethane. Hexane was dropwise added to the benzene solution until a suspension appeared. The suspension was stood for 12 hr at room temperature to give 8.3 g crystalline substance. Yield; 52%, IR (cm^{-1}); 3070 (CH, aromatic), 2928 (CH_3), 2849 (CH_3), 1662 (C=O), 1600 (C=C) 1448 (CH_3), 1352 (C-O-C), 1202 (C-O-C), 1048 (C-O), ^{1}H-NMR($CDCl_3$) δ; 10.63 (1H, s), 8.42-8.37 (2H, q), 7.63-7.35 (2H, q), 7.02 (1H, s), 4.00 (12H, s).

2-Hydroxymethy-1,4,9,10-tetramethoxyanthracene (2-ha-TMA): 2-Formy-TMA 10 g (30.7mmol) dissolved in 100 ml ethanol was put in 250 ml round bottomed flask. Sodium borohydride 1.16 (30.7 mmol) was portion wise added to the solution and stirred for 1 hr. After that, 1% HCl was added to the solution to become pH=6.0. Ethanol was evaporated to yield a yellow mass, which was recrystallized from methanol. Yield; 84.5%, mp; 174.3~175.5, IR (cm^{-1}); 3420 (OH), 2918 (CH_3), 1614 (C=C), 1442 (CH_3), 1358 (C-O-C), 1100 (CHOH), 1040 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 7.36~7.26 (2H, m), 7.48~7.38 (2H, m), 6.83 (1H, s), 4.92 (2H, s, bro), 3.93 (6H, s), 3.90 (3H, s), 3.77 (3H, s), 1.99 (1H, s).

2-Hydroxymethyl-1,4-dimethoxy-9,10-anthraquinone (DMAQ-1): 7.0 g (21.4 mmol) of 2-hydroxymethyl-1, 4,9,10-tetraanthracene was dissolved in 80 ml acetonitrile and cooled to 0~5°C in ice bath. 24.7 g (45 mmol) cerium (IV) diammonium nitrate (CAN) dissolved in 80 ml water was droped into the solution during 30 min and stirred for 2 hr. After the reaction was finished, 100 ml water was added and the extracted with dichlojromethane. The dichloromethane solution was dehydrated with anhydrous sodium sulfate and decanted. The solution was evaporated to a mass, which was purified using silica gel column chromatography with ethyl acetate/hexane (1:1) and recrystallization from methanol. Yield; 81%, mp; 180.2~181.8, IR(cm^{-1}); 3475 (OH), 2919 (CH_3), 1662 (C=O), 1584 (C=C), 1450 (CH_3), 1242 (C-O-C), 1038 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.15~8.05 (2H, m), 7.79~7.69 (2H, m), 7.69 (1H, s), 4.79 (2H, s), 4.02 (3H, s), 3.85 (3H, s).

General method for synthesis of 2-(1-hydroxyalkyl)-1, 4-dimethoxy-9,10-anthraquinone

30.7 mmol of 2-Formyl-1,4,9,10-tetramethoxyanthracene was dissolved in 80 ml tetrahydrofuran. 92 mmol of alkyl magnesium bromide dissolved in 50 ml tetrahydrofuran was added dropwise to the substrate solution during 30 min and stirred for 4 hr. After the reaction was finished, 150 ml of 10% ammonium chloride was added and extracted with dichloromethane. The dichloromethane solution was dehydrated with anhydrous sodium sulfate and decanted. The solution was evaporated to a mass, which was recrystallized from methanol or chromatographed in a silica gel column.

2-(1-Hydroxyethyl)-1,4-dimethoxy-9.10-anthraquinone (DMAQ-2): Yield; 77.3%, mp 129.6~130.2, IR (cm^{-1}); 3475 (OH), 2952 (CH_2), 1664 (C=O), 1650 (C=O), 1584 (C=C), 1540 (C=C), 1450 (CH_2), 1233 (C-O-C), 1035 (C-O), 978, ^{1}H-NMR ($CDCl_3$) δ; 8.12~8.02 (2H, m), 7.68-7.58 (2H, m), 7.50 (1H, s), 5.27 (1H, m), 3.90 (3H, s), 3.80 (3H, s), 2.82 (1H, s, bro), 1.46 (3H, d, J= 6.39 Hz).

2-(1-Hydroxypropyl)-1,4-dimethoxy-,9,10-anthraquinone: Yield; 63.8%, mp 123.1~123.9, IR (cm^{-1}); 3492 (OH), 2962 (CH_2), 2926 (CH_2), 1669 (C=O), 1557 (C=C), 1584 (C=C), 1542 (C=C), 1236 (C-O-C), 1120 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.13~8.03 (2H, m), 7.69~7.59 (2H, m), 7.49 (1H, s), 5.07 (1H, t), 3.92 (3H, s), 3.81 (1H, s), 1.75 (2H, m), 0.99 (3H, t), ^{13}C-NMR ($CDCl_3$) δ; 183.2, 182.6, 156.6, 151.0, 148.4, 134.1, 133.7, 133.5, 133.1, 126.5, 126.4, 126.2, 121.3, 117.2, 69.5, 62.3, 56.5, 31.1, 10.1.

2-(1-Hydroxybutyl)-1,4-dimethoxy-9,10-anthrquinone: Yield; 62.5%, mp 197.7~498.6, IR (cm^{-1}); 3490 (OH), 1690 (C=O), 1662 (C=O), 1582 (C=C), 1462, 1258, 1040 (C-O), 998, 972 ^{1}H-NMR ($CDCl_3$) δ; 8.18~8.08 (2H, m), 7.78~7.68 (2H, m), 7.55 (1H, s), 5.18 (1H, m), 3.98 (3H, s), 3.87 (3H, s), 2.87 (1H, s, bro), 1.77 (2H, m), 1.36~1.25 (2H, m), 0.97 (3H, t), ^{13}C-NMR ($CDCl_3$) δ; 183.3, 182.7, 171.1, 156.7, 150.8, 148.8, 134.2, 133.7, 133.5, 133.1, 126.6, 126.4, 126.3, 121.3, 117.0, 68.1, 62.2, 56.5, 40.4, 20.9, 13.7.

2-(1-Hydroxypentyl)-1,4-dimethoxy-9,10-anthrquinone: Yield; 59%, mp 85.1~86.5, IR (cm^{-1}); 3496 (OH), 2904 (CH_2), 2848, 1662 (C=O), 1586 (C=C), 1540 (C=C), 1456, 1040 (C-O), 975, ^{1}H-NMR ($CDCl_3$) δ; 8.21~8.11 (2H, m), 7.75~7.45 (2H, m), 7.54 (1H, s), 5.16 (1H, m), 3.40 (3H, s), 3.88 (3H, s), 2.58 (1H, s, bro), 1.79~1.61 (2H, m), 1.53~2.56 (4H, m), 0.91 (3H, t).

2-(1-Hydroxyhexyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 58%, mp 87.5~89.2, IR (cm^{-1}); 3490 (0H), 2925 (-CH_2), 2845 (OCH_3), 1662 (C=O), 1654 (C=O), 1584 (C=C), 1542 (C=C), 1322 (C-O-C), 1241 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.14~8.04 (2H, m), 7.70~7.60 (2H, m), 7.50 (1H, s), 5.11 (1H, t), 3.93 (3H, s), 3,82 (3H, s), 2.96 (1H, s, bro), 1.78~1.62 (2H, m), 1.31 (6H, s, bro), 0.84 (t, 3H).

2-(1-Hydroxyheptyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 54.6%, mp 78.5~79.8, IR (cm^{-1}); 3490 (OH), 2925 (-CH_2), 2845 (-CH_2), 1662 (C=O), 1654 (C=O), 1584 (C=C), 1542 (C=C), 1452, 1322 (C-O-C), 1241 (C-O), 1122 (C-OH), ^{1}H-NMR ($CDCl_3$) δ: 8.15~8.05 (2H, m), 7.72~7.62 (2H, m), 7.56 (1H, s), 5.18 (1H, m), 3.93 (3H, s), 3.84 (3H, s), 1.67 (2H, m), 1.30 (8H, m), 0.86 (2H, t), ^{13}C-NMR ($CDCl_3$) δ; 183.0, 182.4, 156.5, 150.6, 149.1, 133.9, 133.4, 133.3, 132.9, 126.3, 126.2, 126.0, 120.9, 116.9, 67.9, 62.0, 60.1, 38.2, 31.4, 28.8, 25.6, 22.3, 20.7, 13.8.

2-(1-Hydroxyoctyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 56.7%, mp 160.9~162.1, IR (cm^{-1}); 3420 (OH), 2950 (-CH_2), 2915 (-CH_2), 2850, 1688 (C=O), 1666 (C=O), 1582 (C=C), 1548 (C=C), 1458, 1318 (C-O-C), 1250 (C-O), 998, 975, ^{1}H-NMR ($CDCl_3$) δ; 8.15-8.04 (2H, m), 7.71~7.61 (2H, m), 7.61 (1H, s), 5.15 (1H, m), 3.93 (3H, s), 3.83 (3H, s), 3.26 (1H, s, bro), 1.78-1.63 (2H, m), 1.25 (10H, s, bro), 0.85 (3H, t).

2-(1-Hydroxynonyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 50%, mp 87.6~88.3, IR (cm^{-1}); 3495 (OH), 2905 (CH_2), 2845, 1662 (C=O), 1652 (C=O), 1585 (C=C), 1542 (C=C), 1320 (C-O-C), 1250 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.17~8.07 (2H, m), 7.73~7.63 (2H, m), 7.63 (1H, s), 5.14 (1H, m), 3.96 (3H, s), 3.85 (3H, s), 2.85 (1H, s, bro), 1.77~1.63 (2H, m), 1.24 (12H, s, bro), 0.85 (3H, t).

2-(1-Hydroxyltridecyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 48.4%, mp 90.2~91.2, IR (cm^{-1}); 3490 (OH), 2905 (CH_2), 2845, 1662 (C=O), 1585 (C=C), 1542 (C=C), 1452 (CH_2), 1322 (C-O-C), 1245 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.17~8.07 (2H, m) 7.73~7.63 (2H, m), 7.63 (1H, s), 5.14 (1H, m), 3.96 (3H, s), 3.85 (3H, s), 2.85 (1H, s, bro), 1.77~1.63 (2H, m), 1.24 (12H, s, bro), 0. 85 (3H, t).

2-(1-Hydroxy-1-cyclohexylmethyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 61.7%, IR (cm^{-1}); 3495 (OH), 2920 (-CH_2), 2848 (-CH_2), 1662 (C=O), 1640 (C=O), 1582 (C=C), 1540(C=C), 1445, 1312 (C-O-C) ^{1}H-NMR ($CDCl_3$) δ; 8.19~8.09 (2H, m), 7.73~7.63 (2H, m), 7.46 (1H, s), 4.91 (1H, d, J=5.31), 3.99 (3H, s), 3.86 (3H, s), 2.47 (1H, s, bro), 1.72 (5H, s, bro), 1.31~1.16 (6H, m)

2-(1-Hydroxy-1-phenylmethyl)-1,4-dimethoxy-9,10-anthraquinone: Yield; 48.9%, mp 184.8~185.4, IR (cm^{-1}); 3445 (OH), 3050 (C-H, aromatic), 1662 (C=O), 1580 (C=C), 1545 (C=C), 1445, 1250 (C-O-C), 1038, 978, ^{1}H-NMR ($CDCl_3$) δ; 8.20~8.10 (2H, m), 7.75~7.65 (3H, m), 7.36 (5H, s), 6.21 (1H, d, J=4.14 Hz), 4.04 (3H, s), 3.58 (3H, s), 2.76 (1H, d, J=4.41).

General method for synthesis of 2-(1-hydroxylalkyl)-1,4-dihydroxy-9,10-anthraquinones

15 mmol of 2-(1-Hydroxymethy)-DMAQ was added to 250 ml round-bottomed flask and dissolved in 150ml dichloromethane. 82.5 mol of aluminum chloride was slowly added to the solution and stirred for 3 hr under nitrogen. After the reaction was finished, 10 ml HCl was added to the reaction mixture, diluted with 200 ml water and extracted with dichloromethane. Evaporation of the solvent gave a red solid mass. This was recrystallized from methanol or chromatographed in a silica gel column.

2-Hydroxymethy-1,4-dihydroxy-9,10-anthraquinone (2-hydroxymethylDHNQ): Yield; 38%, mp 162.1~162.7, IR (cm^{-1}); 3440~3100 (OH), 1619 (C=O), 1582 (C=C), 1428, 1100 (-COH), 1042 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 8.39~8.29 (2H, m), 7.91~7.81 (2H, m), 7.56 (1H, d, J= 2.16 Hz), 5.08 (m, 1H), 4.82 (m, 1H). 2.60 (m, 1H).

2-(1-Hydroxyethyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 56.5%, mp 113.0~113.8, IR (cm^{-1}); 3600~3160 (OH), 1620 (C=O), 1580 (C=C), 1420 (CH_2), 1100 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.46 (1H, s), 12.89 (1H, s), 8.39~8.29 (2H, m), 7.88~7.78 (2H, m), 7.46 (1H, s), 5.22 (1H, m), 2.51 (1H, d, J=4.41 Hz), 1.58 (3H, d, J= 6.39 Hz).

2-(1-Hydroxypropyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 53%, mp 162.1~163.2, IR (cm^{-1}); 3550~ 3050 (OH), 2954 (CH2), 2918 (CH2), 1620 (C=O), 1582 (C=C), 1428, 1100 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.44 (1H, s), 12.88 (1H, s), 8.38~8.28 (2H, m), 7.87~7.77 (2H, m), 7.42 (1H, s), 4.95 (1H, s), 2.48 (1H, d, J=5.49 Hz), 1.98~1.83 (2H, m), 1.03 (3H, t).

2-(1-Hydroxybutyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 53%, mp 132.1~133.7, IR (cm^{-1}); 3600~3200 (OH), 2950 (CH_2), 1620 (C=O), 1582 (C=C), 1422, 1108 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.33 (1H, s), 12.79 (1H, s), 8.29~8.19 (2H, m), 7.83~7.73 (2H, m), 7.37 (1H, s), 5.05 (1H, m), 2.66 (1H, d, J=5.13 Hz), 1.75~ 1.46 (4H, m), 0.98 (3H, t).

2-(1-Hydroxypentyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 52%, mp 127.8~128.6, IR (cm^{-1}); 3600~ 3150 (-OH), 2945 (-CH_2), 2908 (-CH_2), 2849 (-CH_3), 1618 (C=O), 1578 (C=C), 1238 (-OH), 1102 (C-OH), ^{1}H-NMR ($CDCl_3$) δ; 13.33 (1H, s), 12.88 (1H, s), 8.38-8.28 (2H, m), 7.82~7.72 (1H, m), 7.38 (1H, s), 5.04 (1H, m), 2.5 (1H, d, J=), 1.88~1.76 (2H, m), 1.62~1.33 (4H, m), 0.92 (3H, t).

2-(1-Hydroxyhexyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 54%, mp 140.5~141.2, IR (cm^{-1}); 3550~ 3110 (OH), 2950 (-CH_2), 2925 (-CH_2), 1618 (C=O), 1578 (C=C), 1422 (-CH_2), 1340 (C-O-C), 1250 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.36 (1H, s), 12.83 (1H, s), 8.31~ 8.21 (2H, m), 7.83~7.73 (2H, m), 7.37 (1H, s), 5.01 (1H, t), 2.39 (1H, s, bro), 1.81~1.45 (8H, m), 0.96 (3H, t).

2-(1-Hydroxyheptyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 49%, mp 102.4~104.9, IR (cm^{-1}); 3550-3110 (OH), 2950 (-CH_2), 2925 (-CH_2), 2845, 1618 (C=O), 1578 (C=C), 1422, 1340 (C-O-C), 1250 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.59 (1H, s), 13.04 (1H, s), 8.54~ 8.44 (2H, m), 7.59 (1H, s), 5.19 (1H, m), 2.78 (1H, d, J=5.13 Hz), 1.98 (2H, m), 1.54 (8H, s, bro, 1.09 (3H, t), ^{13}C-NMR ($CDCl_3$) δ; 187.2, 186.2, 157.9, 155.4, 146.4, 134.5, 134.3, 133.4, 133.3, 127.0, 125.7, 112.3, 111.5, 69.3, 36.8, 31.7, 29.1, 25.7, 22.6, 14.0.

2-(1-Hydroxyoctyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 49%, mp 178.8, IR (cm^{-1}); 3600~3150 (OH), 2950 (-CH_2), 2915 (-CH_2), 2850, 1620 (C=O), 580 (C=C), 1430, 1268 (C-O), 1238 (C-O), 1105 (C-OH), ^{1}H-NMR ($CDCl_3$) δ; 13.45 (1H, s), 12.89 (1H, s), 8.39~ 8.29 (2H, m), 7.88~7.78 (2H, m), 7.43 (1H, s), 5.05 (1H, t), 2.40 (1H, d, J=5.50), 1.77 (2H, m), 1.56~1.30 (10H, m), 0.88 (3H, t).

2-(1-Hydroxynonyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 47%, mp 91.5~92.6, IR (cm^{-1}); 3620~3150 (OH), 2905 (CH_2), 2848, 1620 (C=O), 1582 (C=C), 1242 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.44 (1H, s), 12.89 (1H, s), 8.38~8.28 (2H, m), 7.87~7.77 (2H, m), 5.03 (1H, m), 2.47 (1H, d, J=5.58), 1.78 (2H, m), 1.26 (12H, s, brod) 0.87 (3H, t).

2-(1-Hydroxytridecyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 42%, mp 78.8~79.6, IR (cm^{-1}); 3620~ 3150 (OH), 2905 (CH_2), 2848, 1620 (C=O), 1582 (C=C), 1425, 1242 (C-O), ^{1}H-NMR ($CDCl_3$) δ; 13.44 (1H, s, OH), 12.89 (1H, s, OH), 8.38~8.28 (2H, m), 7.87~ 7.77 (2H, m), 7.42 (1H, s), 5.03 (1H, m), 2.47 (1H, d, J=5.58 Hz, OH), 1.58 (2H, m), 1.26 (20H, s, brod), 0.87 (3H, t).

2-(1-Hydroxy-1-cyclohexymethyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 55.6%, mp 178.7~179.8, IR (cm^{-1}); 3600~3250 (OH), 2908 (-CH_2), 2845 (-CH_2), 1618 (C=O), 1582 (C=C), 1445 (-CH_2), 1242 (C-O), 1100, ^{1}H-NMR ($CDCl_3$) δ; 13.45 (1H, s), 12.92 (1H, s), 8.38~8.28 (2H, m), 7.89~7.79 (2H, m), 7.50 (1H, s), 4.91 (1H, m), 4.4 (1H, d, J=5.31 Hz), 1.69 (6H, s, bro), 1.20 (5H, s, bro), ^{13}C-NMR ($CDCl_3$) δ; 186.6, 185.7,

157.2, 155.2, 146.7, 134.0, 133.8, 133.0, 132.9, 126.3, 126.2, 111.5, 110.8, 70.8, 29.2, 26.5, 25.8, 25.5.

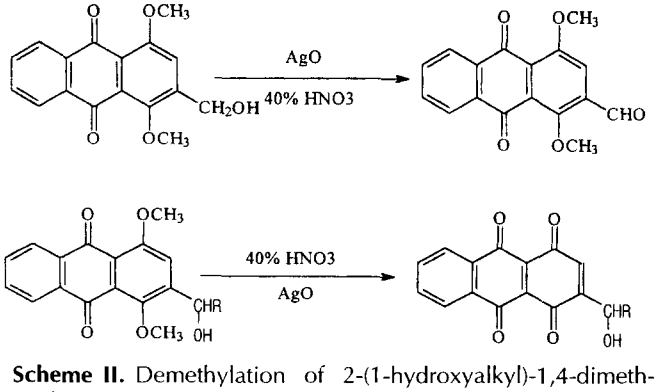
2-(1-Hydroxy-1-phenymethyl)-1,4-dihydroxy-9,10-anthraquinone: Yield; 38%, mp 221.5~222.8, IR (cm^{-1}); 3600~3200 (OH), 3050 (CH, aromatic), 1662 (C=O), 1580 (C=C), 1340 (C-O-C), 1235 (C-O), 1160 (C-OH), ^{1}H-NMR ($CDCl_3$) δ; 13.41 (1H, s), 12.88 (1H, s), 8.30~8.29 (2H, m), 7.85~7.75 (2H, m), 7.52 (1H, s), 7.46~7.26 (5H, m), 6.17 (1H, d, *J*=4.05 Hz), 2.84(1H, d, *J*= 4.41 Hz).

RESULTS AND DISCUSSION

Chemistry

1,4,9,10-tetramethoxyanthracene was formylated by Vilsmeier's method to obtain 2-formyl-1,4,9,10-tetramethoxyanthracene (yield, 52%). This underwent Grignard reaction with various alkylmagnesium halides to give 2-(1-hydroxyalkyl)-1,4,9,10-tetramethoxy anthracene derivatives. These derivatives were oxidatively demethylated, resulting in the formation of 2-(1-hydroxyalkyl)-1,4-dimethoxy-9,10-anthraquinone (DMAQ) derivatives (yield; 50~77%). 2-(1-Hydroxyalkyl)-DMAQ derivatives were demethylated with aluminium chloride to produce 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinone (DHAQ) derivatives (yield, 50~80%). AgO/HNO_3 as a recommended demethylating agent (terada *et al.*, 1987) failed to produce these 1,4-dihydroxyanthraquinones. Oxidation of 2-(1-hydroxymethyl)-DMAQ and 2-(1-hydroxytridecyl)-DMAQ with this reagent gave 2-formyl-1,4-dimethoxy-9,10-anthraquinone and 2-(1-hydroxytridecyl)-1,4,9,10-anthradiquinone, respectively (Scheme II).

Selective acylation of 2-(1-Hydroxyalkyl)-DMAQ and -DHNQ derivatives at C-1' with various organic acids in the presence of dicyclohexylcarbodiimide/4-dimethylaminopyridine provided 2-(1-acyloxyalkyl)-DMAQ and -DHAQ derivatives, respectively. The hindered acylation of phenolic OH groups may be evidently due to the strong hydrogen bonding between quinonoid carbonyls and phenolic OH groups.



Scheme II. Demethylation of 2-(1-hydroxyalkyl)-1,4-dimethoxyl-9,10-ananthraquinones

Cytotoxic activity and antitumor action

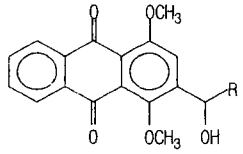
The cytotoxic activity of 2-(1-hydroxyalkyl)-DMAQ derivatives against L1210 cells was summarized in Table I. Only anthraquinones having shorter side chains (C_1 -C_5) exhibited a moderate cytotoxicity (ED_{50}, 25~80 μg/ml), while those with more than five C-atoms were practically inactive (ED_{50}, >100 μg/ml).

Next, the ED_{50} of 2-(1-hydroxyalkyl)-DHAQ derivatives were determined and compared to that of 2-(1-hydroxyalkyl)-DMAQ derivatives. 2-(1-Hydroxyalkyl)-DHAQ derivatives exhibited stronger cytotoxic activity than 2-(1-hydroxyalkyl)-DMNQ derivatives. The DHAQ derivatives possessing the side chain longer than eight C-atoms showed no significant cytotoxic activity.

The mechanism of the cytotoxic activity of the DHAQ derivatives are supposed to be free radical formation for production of superoxide and bioreductive activation as well as DNA intercalation of the DHAQ series.

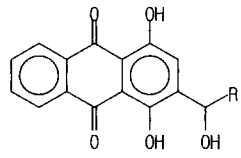
2-(1-Hydroxyethyl)-DHAQ(DHAQ-14) could undergo a redox equilibrium in L1210 cells, though weak (Scheme III). First, the anthradiquinone as a Michael acceptor would undergo an electrophilic reaction with cellular nucleophiles. Action mechanism of mitoxantrone is a good example for such a biooxidative activaton (Ehninger *et al.*, 1990; Mewes *et al.*, 1993; Blanz *et al.*, 1991). Secondly, the resulted anion radical shifts its radical electron to oxygen to form superoxide, which involves in oxidative stress, which is a well-known cytotoxic mechanism of quinones (Jeziore *et al.*, 1993; Fisher *et al.*, 1992, 1990; Morier-Teissier E *et al.*, 1990; Dodd and Mukherjee, 1984). Third, its anionic electron pair migrate to side chain ensuing, after elimination of H_2O, an quinone methide as a

Table I. Cytotoxic effect of 2-(1-hydroxyalkyl)-1,4-dimethoxy -9,10-anthraquinone against L1210 cells



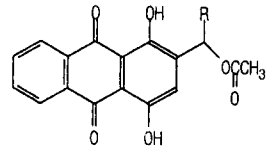
No.of compd,	R-	ED_{50} (L1210), μg/ml
DMAQ-1	H	45
DMAQ-2	Methyl	24.8
DMAQ-3	Ethyl	50
DMAQ-4	Propyl	56
DMAQ-5	Butyl	80
DMAQ-6	Pentyl	>100
DMAQ-7	Hexyl	>100
DMAQ-8	Heptyl	>100
DMAQ-9	Octyl	>100
DMAQ-10	Dodecyl	>100
DMAQ-11	Cyclohexyl	>100
DMAQ-12	Phenyl	>100

Table II. Antitumor effect of 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinone derivatives



No.of compd,	R-	L1210	Sarcoma 180	
		ED_{50} (μg/ml)	μmol/kg/day	T/C (%)
DHAQ-13	H	15	4.3 (16)	125.4
DHAQ-14	Methyl	1.9	4.5 (16)	139.2
DHAQ-15	Ethyl	7.2	4.8 (16)	135.1
DHAQ-16	Propyl	10.2	5.0 (16)	125.3
DHAQ-17	Butyl	23.7	5.2 (16)	110.1
DHAQ-18	Pentyl	58.0	5.5 (16)	108.5
DHAQ-19	Hexyl	60	5.7 (16)	115.1
DHAQ-20	Heptyl	>80	5.9 (16)	103.1
DHAQ-21	Octyl	>80	6.1 (16)	98.8
DHAQ-22	Dodecyl	>80	7.0 (16)	101.4
DHAQ-23	Cyclohexyl	>80	5.6 (16)	92
DHAQ-24	Phenyl	5.6	5.5 (16)	137.5

Table III. Antitumor effect of 2-(1-acetyoxyalkyl)-1,4-dihydroxy-9,10-anthraquinone derivatives

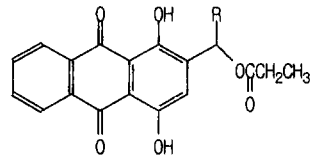


No	R-	L1210 cell	S-180	
		ED_{50} (μg /ml)	μmol/kg/day	T/C (%)
DHAQ-25	H	2.75	16	136
DHAQ-26	Methyl	1.42	16	146
DHAQ-27	Ethyl	6.51	16	142
DHAQ-28	Propyl	11	16	135
DHAQ-29	Butyl	34	16	108
DHAQ-30	Pentyl	>100	16	101
DHAQ-31	Hexyl	>100	16	
DHAQ-32	Heptyl	>100	16	
DHAQ-33	Octyl	>100	16	
DHAQ-34	Dodecyl	>100	16	
DHAQ-35	Cyclohexyl	>100	16	
DHAQ-36	Phenyl	3.5	16	

reactive Michael acceptor. Fourth, DNA intercalation, a proved cytotoxic mechanism of anthraquinone derivatives (Koyama *et al.*, 1989; Palmer *et al.*, 1988; Baguley B. C., 1991) is expected to be one of the action mechanisms. The cellular nucleophiles such as DNA and enzymes of a cell add to the Michael acceptor to form a covalent bond resulting in damage of it. The elimination reaction has to obey the Sayzeff rule, Where presence of methyl group (R=methyl) accelerates the formation of the quinone methide more than hydrogen (R=H). In fact DHAQ-13 showed less activity than DHAQ-14. However, a longer R (>heptyl) expected to retard the elimination reaction, resulting in lower cytotoxicity. Further evidence for the bioreductive activation could be brought from the enhanced cytotoxic activity of 2-(1-acetyloxyalkyl)-DHAQ derivatives. Replacement of 1'-hydroxy group by acetoxy group as a better leaving group, has to accelerate the formation of the quinone methide resulting in enhancement of the cytotoxic activity. For example, the acetylated DHAQ-25 (ED_{50}, 2.75 μg/ml) was 6 times more cytotoxic in L1210 cells than the unacetylated DHAQ-13 (ED_{50}, 15 μg/ml). In consequence, it is supposed that the cytotoxicity of 2-(1-oxyalkyl)-DHAQ derivatives with shorter side chain could be mediated by all three mechanismes, whereas the cytotoxicity of 2-(1-oxyalkyl)-DMAQ derivatives without free phenolic groups does not involve in the bioreductive activation, and thus could result probably from DNA intercalation, or the oxidative stress.

It was expected that introduction of phenyl moiety in the side chain may affect the cytotoxicity. It is remarkable that the compound **24** containing phenyl

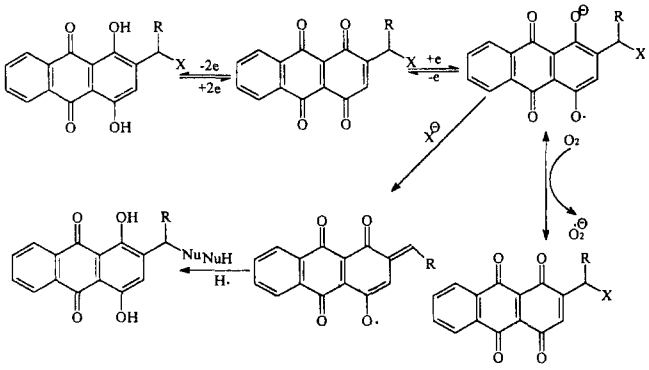
Table IV. Antitumor effect of 2-(1-propanoyloxyalkyl)-1,4-dihydroxy-9,10-anthraquinone derivaties



N o ofcompd.	R-	L1210 cell	S-180	
		ED_{50} (μg/ml)	μmol/kg/day	T/C (%)
DHAQ-37	H	50	16	120
DHAQ-38	methyl	38	16	115
DHAQ-39	ethyl	46	16	110
DHAQ-40	propyl	>100	16	103
DHAQ-41	butyl	>100	16	102
DHAQ-42	pentyl	>100	16	98
DHAQ-43	hexyl	>100	16	
DHAQ-44	heptyl	>100	16	
DHAQ-45	octyl	>100	16	
DHAQ-46	dodecyl	>100	16	
DHAQ-47	cyclohexyl	>100	16	
DHAQ-48	phenyl	>100	16	

group in the side chain showed stronger cytotoxicity than the compound **23** with cyclohexyl group at the same position (Table II). Although further studies are required, it is assumed that an aromatic moiety in the side chain plays an important role in the binding of the compound to the receptor molecule such as cell surface receptors, enzymes or nucleic acids.

Next, the effect of acetylation at C-1' was examined. As shown in Table III, 1'-acetylation enhanced the cytotoxicity remarkably, while 1'-propionylation rather



Scheme III. Proposed mechanism of bioreductive activation on 2-substituted 1,4-dihydroxy-9,10-anthraquinone.

showed a reverse effect (Table IV). In an earlier study [Back *et al.*, Arch.der Pharmazie (Weinheim) in press] on the cytotoxicity of 5,8-dihydroxy-1,4-naphthoquinone derivatives, it was proposed that acetyl group, bioisosteric with a double bond in the side chain shikonin, would contribute to potentiation of the cytotoxicity by enhancing the binding with the receptors as well as increasing the electrophilicity of the anthraquinone ring to cellular nucleophiles. In this sense, it is supposed that the cytotoxicity-enhancing effect of the acetyl group in the side chain of DHAQ derivatives in addition to the bioreductive activation as mentioned above, is due to strengthening of its binding to a receptor and enhancement of electrophilic reactivity of the anthraquinone ring. Meanwhile, the introduction of propionyl group in the DHAQ structure worsened the water solubility, resulting in decrease of cytotoxicity (Table IV).

Prolongation of life span of ICR mice bearing S-180 cells

Subsequently, the T/C values of 2-(1-oxyalkyl)-DHAQ derivatives were measured and the results were summarized in Table II, III, IV. All of 2-(1-oxyalkyl)-DMAQ derivatives (Table I) showed no activity. As shown in Table II, the cytotoxicity and T/C value of the compounds 13-16 possessing shorter side chains could be correlated; they possessed greater cytotoxicity (ED_{50}, 9~10.2 μg/ml) and higher T/C value (T/C, 125~139%). For instance, DHAQ **14** with the most cytotoxic activity has the highest T/C value. Meanwhile, the compounds **17-23** with side chain of more than five carbon atoms showed lower cytotoxicity and antitumor action. The effect of phenyl ester group in the side chain was also pronounced for the antitumor activity; the compound **24** showed the highest T/C value of 138% among the compounds in Table II. In contrast, the compounds with cyclohexyl moiety had an inactive T/C value of 98%. It is suggested that the presence of phenyl group in the side chain of the derivatives is very important not only for the cytotoxicity, but also for the antitumor activity partially due to enhancement in binding to receptor. Meanwhile, a larger acyl group might afford a low water solubility, resulting in decrease of cytotoxicity.

Taken together, the lipophilicity and the electrophilicity of the anthraquinone ring seem to be important for the enhancement of antitumor activity of the DHAQ derivatives.

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