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Full Length Research Paper

Studies of the anticancer effect of sesquiterpene lactone from *Carpesium rosulatum*

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In search for plant-derived anticancer compound, it was found that the chloroform extracts obtained from the whole plant of *Carpesium rosulatum* MIQ. (Compositae) exhibited significant anticancer activity against human tumor cell line, A549, SK-OV-3, SK-MEL-2, XF-498, HCT-15. The structures and stereochemistry of these compounds were established on the basis of analysis of spectra including mp, $[\alpha]_D^{25}$, IR, UV, EI-MS, MS, ^{1}H-NMR, ^{13}C-NMR and some chemical transformations as follows: 1 (4β,10α-dihydroxy-guaia-8α,12-olide), 2 (4β,10α-dihydroxy-1(2),11(13)-guaiadien-8α,12-olide), 3 (3β,8β-dihydroxy-1α,5α-guaian-10(14)-ene-6α,12-olide). Anticancer activity of compounds obtained from *C. rosulatum* MIQ. on five tumor cells line was evaluated by procedure of SRB methods. 2 significantly effects to the five human tumor cell lines.

Key words: *Carpesium rosulatum,* anticancer activity, human tumor cell lines, SRB.

INTRODUCTION

Carpesium rosulatum (Inuleae, Compositae), uncommon in Korea distributed in South Korea, has been used in Korean traditional medicine for its antipyretic, analgesic, vermifugic and anti-inflammatory properties (Yook, 1981). Seeds of Carpesium species are explored as a traditional expellent of seat worms in Japan (Maruyama et al., 1995). Previous works on Carpesium species report the occurrence of several germacranolides and triterpenoids in *C. divaricatum* as well as the determination of biological activities including cytotoxicity assays (Kim et al., 1997).

Maruyama et al. reported the isolation of several sesquiterpene lactones from the genus Carpesium; granilin (Maruyama and Shibata, 1975), carpesiolin (Maruyama and Shibata, 1975), carabrone (Maruyama and Omura, 1977), carabrol (Maruyama et al., 1983) and ivaxillin (Maruyama et al., 1983). Recently, thymol compounds from aerial parts of Carpesium species have been evaluated for cancer treatment in South Korea (Zee et al., 1998).

A huge reservoir of bioactive compounds exists in the over 400 000 species of plants on Earth, only a small percentage of which have been examined in research studies. Plants have been and continue to be an important source of anticancer agents (Newman et al., 2002). Worldwide efforts are ongoing to identify new anticancer compounds from plants.

The approaches for selecting plants to be tested for new bioactive compounds vary from random selection to more guided selection strategies such as the ethnopharmacological approach (Cox, 1994).

This latter strategy utilizes knowledge gained from the folk medicinal uses of a plant to guide the selection process. This approach generally increases the chance of finding active compounds compared with the random selection strategy.

To the best of our knowledge, extracts prepared from *Carpesium* spp. have not been evaluated for anticancer properties. Continuing the investigations and our research on the pharmacologically active compounds, we report here the isolation and structure identification of sesquiterpene as well as some source plants where potential anticancer activity could be characterized. In this paper, isolation of the chloroform fractions from whole plants of *C. rosulatum* yielded three sesquiterpene compounds.

The structure of the compound was determined by chemical analyses as well as nuclear magnetic resonance spectroscopy. The 2 exhibited strong anticancer activity *in vitro*.

Table 1. The antiproliferative effect of organic solvent fractions from *C. rosulatum* MIQ.

	IC_{50} values*				
Fractions	**A549**	**SK-OV-3**	**SK-MEL-2**	**XF498**	**HCT15**
$CHCl_3$	22.6	34.6	20.7	24.0	22.9
CR1	13.4	12.3	14.5	14.4	12.6
CR2	20.0	20.0	14.0	20.0	16.2
CR3	13.8	13.3	15.9	15.5	12.5
CR4	20.0	17.2	20.0	20.0	20.0
CR5	20.0	20.0	15.3	20.0	20.0
CR1.1	20.0	20.0	20.0	20.0	20.0
CR1.2	11.4	20.0	19.5	15.9	12.3
CR1.3	7.6	6.8	7.4	10.2	11.9
CR1.4	17.6	20.0	18.6	20.0	20.0
CR1.5	20.0	20.0	20.0	20.0	20.0

* IC_{50} value of compound against each cancer cell line, which was defined as a concentration, (μM) that caused 50% inhibition of cell growth *in vitro* , A549 : non small cell lungcarcinoma, SK-OV-3 : adenocarcinoma, ovary malignant ascites, SK-MEL-2 : malignant melanoma, metastasis to skin of thigh, XF498 : central nerve system tumor, HCT15 : colon adenocarcinoma.

MATERIALS AND METHODS

Plant materials and isolation of active compounds

C. rosulatum (Inuleae, Compositae), collected at Mt. O-de, Kangwondo, Korea in August 2000, was identified by Prof. Seung-Jo Yoo. A voucher specimen (No. 2000-0815-2101) has been deposited in the Pharmacy Herbarium of the Sung Kyun Kwan University (Suwon, Korea). For anticancer bioassay from methanol extract, two sets (50 g each) of roughly ground air-dried plant materials of every selected species were extracted twice separately with methanol (200 ml for each extraction) by refluxing for 4 h on a sonication bath at 35°C. For the bioassay, the fractions were dissolved with 3 mg/ml in dimethyl sulfoxide and further diluted with incubation buffer. These were again pooled by methanol extracts, of which n-hexane, chloroform, ethylacetate and butanol fractions of *C. rosulatum* was the most active (Table 1). Attempts were then made to isolate the active components from the chloroform-soluble fraction. The air-dried plant material (1.0 kg) was finely ground and extracted at room temperature with MeOH (2 L×3) for 2 weeks. The resulting MeOH extract (103 g) was suspended in H_2O (500 ml) and partitioned with n-hexane (1L×2), chloroform (1L×2), ethylacetate (1 L×2) and n-butanol (1L×2), successively, to give n-hexane (52 g)-, chloroform (10 g)-, ethylacetate (3 g)-, butanol (8 g)- and H_2O (32 g)-soluble fractions. The most active chloroform fraction was applied to a silica gel column and eluted with hexane–EtOAc mixtures of increasing polarity (5:1 - 1:5) to give five subfractions whose main subfraction (subfraction 2; 2.3 g) was chromatographed with silica gel eluted with chloroform/EtOAc (15:1) followed by CH_2Cl_2/EtOAc (9:1) to give five fractions(CR1~CR5). CR3.2 (432 mg) was purified by Lobar A (Merck; CH_2Cl_2/EtOAc, 9:1 ~ 7:3) to yield three main 1 (3 mg), 2 (2.6 mg), 3 (6 mg). Three compounds were shown in Figure. 1.

Compound 1: Colorless oil; $[\alpha]_D^{25}$ -18.58 (MeOH, c 1.0); $UV\lambda_{max}$ (MeOH) nm : 206.8; EI-MS(70eV) [m/z] (rel. int. %): 248[M^+-H_2O, 28), 230(14.8), 95(100); ^{1}H-NMR(600MHz, CD_3OD, δppm): 1.17(3H, s, H-15), 1.21(1H, H-6), 1.28 (3H, s, H-14), 1.58(1H, H-3 and H-5), 1.65(1H, H-2 and H-3), 1.81(1H, H-2), 1.95(1H, H-1), 2.03(1H, dd, *J*=2.8, 11.6, H-9), 2.34(1H, dddd, H-6), 2.41(1H, dd, *J*=1.7, 14.5, H-9), 2.59(1H, m, H-7), 4.35(1H, t, H-8), 5.56(1H, d, *J*=3.0, H-13β), 6.07(1H, d, *J*=3.0, H-13α), ^{13}C-NMR(150MHz, CD_3OD, δppm): 22.8(C-15), 24.6(C-2), 25.1(C-14), 30.2(C-6), 41.7(C-3), 49.6(C-9), 51.0(C-5), 52.2(C-7), 53.2(C-1), 73.9(C-10), 81.1(C-4), 81.2(C-8), 119.0(C-13), 142.4(C-11), 172.4(C-12)

Compound 2: Yellowish crystal; mp: 205 ~ 207°C; $[\alpha]_D^{25}$ -8.73。(MeOH, c 0.5); $UV\lambda_{max}$ (MeOH) nm: 206.5; EI-MS(70eV) [m/z] (rel. int. %): 264[M^+, 6), 246(100), 231, 228, 203, 188, 93, 53; ^{1}H-NMR(600MHz, CD_3OD, δppm): 1.05(1H, q, H-6), 1.37(3H, s, H-15), 1.39 (3H, s, H-14), 2.11(1H, dd, *J*=2.64, 11.4, H-9), 2.23(1H, dd,*J*=3.24, 16.8, H-3), 2.27(1H, td, *J*=12.54, H-6), 2.33(1H, dd, *J*=2.94, 12.48, H-5), 2.38(1H, dd, *J*=1.44, 14.1, H-9), 2.45(1H, H-3), 2.73(1H, m, H-7), 4.01(1H, t, H-8), 5.57(1H, d, *J*=3.12, H-13β), 5.79(1H, q, H-2), 6.07(1H, d, J=3.0, H-13α), ^{13}C-NMR(150MHz, CD_3OD, δppm): 24.3(C-15), 31.9(C-14), 32.3(C-6), 45.5(C-3), 51.3, (C-7), 56.9(C-5), 72.9(C-10), 82.1(C-8), 83.1(C-4), 19.3(C-13), 125.3(C-2), 142.3(C-11), 155.1(C-1), 172.4(C-12)

Compound 3: White amorphous powder; mp: 198℃; $[\alpha]_D^{25}$ -30.21。(MeOH, c 0.15); IR v_{max} cm^{-1}(KBr): 3439(-OH); 1736(γ-lactone moiety), 1720(C=O), 1650(C=C); FAB-MS (rel. int. %): 359[M^++H+glycerol]$^-$(100), 267[M^++H]$^-$, 249[M^++H-H_2O]$^-$, 231[M^++H-2H_2O]$^-$; ^{1}H-NMR(600 MHz, pyridine-d_5, δppm): 1.29(1H, d, *J*=2.64, H-13), 1.47(1H, d, *J*=2.6, H-15), 1.89(1H, dq, H-5α), 2.06(1H, ddd, *J*=7.9, H-7α), 2.11(1H, ddd, *J*=7.9, H-2β), 2.23(1H, dq, H-4α), 2.32(1H, dd, H-9β), 2.37(1H, ddd, *J*=13.0, 8.0, H-2α), 2.82(1H, br, H-1α), 2.88(1H, dd, *J*=13.2, H-9α), 3.24(1H, dq, H-11α), 3.96(1H, dt, *J*=7.7 H-3α), 4.20(1H, dt, *J*=8.8, H-8α), 4.64(1H, t, H-6β), 5.18(1H, br, H-14B), 5.27(1H, br, H-14A), ^{13}C-NMR(150MHz, pyridine-d_5,, δppm): 12.5(C-13), 18.4(C-15), 37.0(C-11), 39.8(C-2), 42.8(C-1), 45.6(C-9), 47.6(C-4), 52.0(C-5), 56.4(C-7), 63.8(C-8), 78.0(C-3), 81.0(C-6), 114.6(C-14), 144.9(C-10), 179.0(C-12).

Cell lines and culture

The following cancer cell lines were used in this study: A549 (non small cell lungcarcinoma), SK-OV-3 (adenocarcinoma, ovary malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nerve system tumor) and HCT15 (colon adenocarcinoma). All lines were maintained in 90% DMEM supplemented with 2 mm l-glutamine, penicillin (100 IU/mL), streptomycin (100 μg/mL) and 10% heat-inactivated fetal bovine

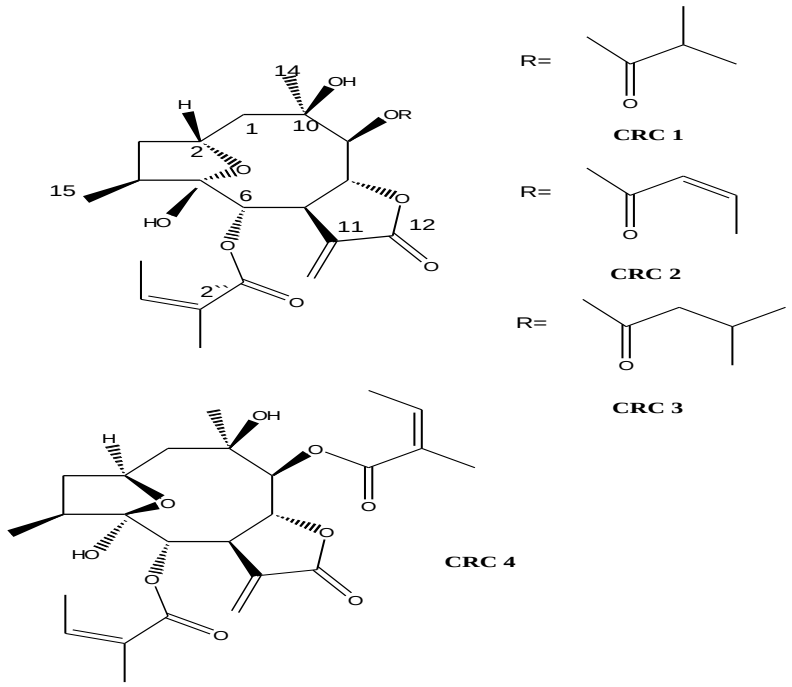

Figure 1. Sesquiterpene lactones from *C. rosulatum* MIQ.

serum. Cells at 70–80% confluence were used for plating for growth inhibition assays (Li et al., 2002).

Antiproliferative assay

Cells were plated in 96-well flat bottom plates at 5000–10000 cell/well. The difference in cell numbers plated adjusts for differences in the growth rates of the various cell lines. Cells were allowed to adhere to the wells overnight, then the herbal extracts were added to triplicate wells in serial 3-fold dilutions. Water was added to the control wells at a 1:10 dilution in medium. These plates were incubated at 37 °C, 5% CO_2 for 3 days, then assayed from growth inhibition using a sulforhodamine B (SRB) assay (Skehan et al., 1990). The cells were fixed by the addition of cold 50% trichloroacetic acid to a final concentration of 10%. After a 1 h incubation at 4 °C, the cells were washed five times with deionized water. The cells were then stained with 0.4% SRB (Sigma) dissolved in 1% acetic acid for 15 to 30 min and subsequently washed five times with 1% acetic acid to remove unbound stain. After the plates had air dried at room temperature, the bound dye was solubilized with 10 mm Tris base and the plates were analysed on a microplate reader (Bio Red, U.S.A.) at 595 nm. The percent growth inhibition was calculated as: (ave. OD control wells – ave. OD herbal extract and compound wells)/(ave OD control wells).

RESULTS AND DISCUSSION

The molecular formula of compound 1 was determined as 4β,10α-dihydroxy- guaia-8α,12-olide by ESIMS and NMR data. Since the NMR data of 1 were not available from the previous literature (Kim et al., 2002). All the signal assignments in ^{1}H and ^{13}C NMR spectra are herein provided. However, Compound 2 was identified as 4β,10α-dihydro-xy-1(2),11 (13)-guaiadien -8α,12-olide by NMR and ESIMS. Its spectral ata are in good agreement with those reported (Kim et al., 2002). Compound 3 was identified as 3β,8β-dihydroxy-1α,5α-guaian-10(14)-ene-6α,12-olide by NMR and ESIMS. Its spectral ata are in good agreement with those reported (Kim et al., 2002). 1, 2 and 3 with the positive control were tested *in vitro* for their antiproliferative activities against five cancer cell lines A549 (non small cell lungcarcinoma), SK-OV-3 (adenocarcinoma, ovary malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nerve system tumor) and HCT15 (colon adenocarcinoma). The growth of five cancer cells was greatly inhibited by various concentrations of 2 in a dose dependent manner (Table 2). The highest tested concentration of 7.6 uM could inhibit cell growth of approximately 50% for SK-MEL-2. The IC50 values of 2, causing 50% cell growth inhibition, are 8.84 uM, 9.14 uM, 7.69 uM, 10.1 uM and 7.74 uM for A549, SK-OV-3, SK-MEL-2, XF498 and HCT15, respectively. The positive control (adriamycin) gave IC_{50} values of 1.61 uM, 2.53 uM, 1.53 uM, 1.23 uM and 4.43 uM to the corresponding cell lines of for A549, SK-OV-3, SK-MEL-2, XF498 and HCT15, respectively. However, 1 and 3 were common inhibitor of cell growth compared with adriamycin, showing only about 50% inhibition of cell growth for 15~30uM. The antiproliferative bioactivity of the 2 may be due to the double bond. sesquiterepne with such a functional group were reported as having a wide range of

Table 2. The antiproliferative effect of compounds from *C. rosulatum* MIQ.

	IC_{50} values*				
Compounds	**A549**	**SK-OV-3**	**SK-MEL-2**	**XF498**	**HCT15**
CRC 1	15.3	11.5	8.10	13.5	10.4
CRC 2	10.8	10.1	6.01	10.5	8.93
CRC 3	9.19	10.1	7.00	9.06	7.54
CRC 4	24.6	26.7	23.2	19.6	21.9
Adriamycin	1.61	2.53	1.53	1.23	4.43

* IC_{50} value of compound against each cancer cell line, which was defined as a concentration (μmol) that caused 50% inhibition of cell growth *in vitro,* A549 : non small cell lungcarcinoma, SK-OV-3 : adenocarcinoma, ovary malignant ascites, SK-MEL-2 : malignant melanoma, metastasis to skin of thigh, XF498 : central nerve system tumor, HCT15 : colon adenocarcinoma.

antifeedant, antifungal, bacteriocidal activities (Kim et al., 1997). To our knowledge, this is the first report on the antitumor activity of the sesquiterepne isolated from *C. rosulatum*. The results indicated that 2, a potent anticancer agent, may be a candidate for further study. However, the molecular mechanism of how 2 inhibits cell growth is not known, but it is being investigated by our research group.

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