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Antitumor Activity of *Psoralea corylifolia*

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Abstract □ The activity-oriented fractionation of *Psoralea corylifolia* led to an isolation of a (+)-bakuchiol **1** as an active principle of its antitumoral property *in vitro*. **1** was observed to exhibit a mild cytotoxicity against five kinds of cultured human cancer cell lines, *i.e.* the A549, SK-OV-3, SK-MEL-2, XF498 and HCT15. The synthesized 2,3-epoxide of (+)-bakuchiol **3** showed the similar activity as the (+)-bakuchiol **1**, whereas the other oxidation derivatives **4** and **5** including the acetyl-(+)-bakuchiol **2** showed a decreased activity.

Keywords □ *Psoralea corylifolia,* Leguminosae, bakuchiol, antitumoral.

Fifty species of oriental medicinal plants had been examined for the antitumoral activity *in vitro*, on the basis of the direct cytotoxicity against five kinds of cultured human cancer cell lines, *i.e.*, A549 (non small cell lung), SK-OV-3 (ovarian), SK-MEL-2 (skin), XF498 (CNS) and HCT15 (colon)[1]. On this survey, the chloroform extract of the fruit of *Psoralea. corylifolia* (Leguminosae) was observed to exhibit a marked cytotoxicity against examined human tumour cells. In this paper, we wish to report the isolation of an active principle of the antitumoral property from the *Psoralea corylifolia*, and also the comparison of the antitumoral potency between the isolated active principle **1** and some synthesized analogues **2-5** of **1**.

EXPERIMENTAL METHODS

^{1}H-NMR spectra were run at 300 MHz and ^{13}C-NMR at 75 MHz and recorded by Bruker AM-300. Low resolution MS (70 eV) were taken with a direct inlet and recorded by JMS-DX303 mass spectrometer (JEOL). The cell used for the experiment, *i.e.*, A549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF498 (central nerve system) and HCT15 (colon) were obtained from the National Cancer Institute (NCI) in USA, which were currently used in the NCI's *in vitro* anticancer-drug screening. Stock cultures were grown in T-25 (Falcon) flasks containing 10 m*l* of RPMI-1640 medium with glutamine, sodium bicarbonate and 5% fetal calf serum. Cells were dissociated with 0.25% trypsin and 30 mM 1,2-cyclohexanediaminetetraacetic acid in PBS before transferring for experiment.

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Antitumor test in vitro

All experiments were conducted by the NCI's protocol[2,3]. Experimental cultures were plated in 96-well microtiter plates (Corning) containing 0.15 m*l* of growth medium per well with a cell density of 5×10^3 (A549 and HCT15), 1×10^4 (SK-MEL-2 and XF498) and 2×10^4 (SK-OV-3). It was incubated in 37℃, humidified 5% CO_2 incubator for one day. Then, media were aspirated off and added the test material in triplicate which was dissolved in media at varing concentrations. In case of necessity, the test material was dissolved in small amount of dimethylsufoxide(DMSO), but the final concentration of DMSO in the medium was not exceed 0.5%. And then, the culture was incubated for additional 2 days. After 2 days of continuous drug exposure, the medium was removed by flicking plates over a sink,

and cells attached to the plastic substratum were fixed by gently layering with 0.1 m*l* of cold 10% trichloroacetic acid(TCA). It was incubated at 4℃ for 1 hour followed by wash with tap water five times to remove TCA solution. After being washed and dried at RT overnight, TCA fixed cells were stained with 0.1 m*l* of 0.4% sulforhodamine B(SRB) in 1% acetic acid per well for 30 minutes. At the end of the staining period, SRB supernatant was removed out and the remaining cells were rinsed with 1% acetic acid five times. It was dried until no standing moisture was visible, and then the bound dye in cells was extracted with 0.1 m*l* of 10 mM unbuffered Tris base (pH 10.5) per well by stirring on a gyratory shaker for 5-10 minutes, followed by measured the optical density at 520 nm by MR700 microplate reader (Dynatech Laboratories). Antitumor activity of the test material at varing concentrations was estimated as the net growth % of cells compared with that of control (without test material, net growth=100%). The dose-response curves of test material was constructed and the ED_{50} value was calculated as the concentration of the test material that caused 50% inhibition of cell growth.

Extraction and isolation

The fruit of *Psoralea corylifolia* was purchased at market and 500g of the dried material was extracted with 20 *L* of MeOH by reflux for 4 hours. The extract was concentrated to the volume of 2 *L* and partitioned with an equal volume of *n*-Hexane. The remained MeOH soluble fraction was concentrated to dryness *in vacuo* and was suspended in 4 *L* of water and extracted with the equal volume of EtOAc. Hence, was yielded the Hexane soluble fraction 14.6g, the EtOAc soluble fraction 76.3g and 16.5 g of the remaining water soluble fraction. Each fraction was subjected to examine the antitumoral activity *in vitro*, of which was active the EtOAc fraction (ED_{50} against A549=80 μg/m*l*). One tenth of EtOAc fraction was divided into four sub-fractions such as Fr. A to Fr. D by the silica gel column chromatography using CH_2Cl_2/MeOH by way of gradient elution. Among these, the Fr. A(3.1g) was most active (ED_{50} against A549=30 μg/m*l*), which was further divided into three portions (Fr. A-1 to Fr. A-3) by the same manner, followed by examining the activity. The most active one, Fr. A-1(2.0g, ED_{50} against A549=15 μg/m*l*) showed one single spot under UV light (*Rf* 0.5 on SiO_2 precoated TLC plate, CH_2Cl_2), which was purified by the repeated preparative TLC to give a compound **1**.

Compound 1

(+)-bakuchiol pale yellow liquid. $[\alpha]_D=+32$ ($CHCl_3$, c=1.0) UV: $\lambda_{max}=260$ nm (MeOH), MS: *m/z* (rel. int.); 256 (M^+, 40), 213 (20), 174 (18), 173 (100), 158 (15), 145 (25), 107 (30). ^{1}H-NMR ($CDCl_3$, δ): 1.25 (3H, s, 15-CH_3), 1.55 (2H, m, 5-CH_2-), 1.63 (3H, s, 1-CH_3), 1.72 (3H, s, 18-CH_3), 2.00 (2H, m, 4-CH_2), 5.07 (1H, dd, *J*=17.2, 1.2 Hz, 17α-H), 5.08 ((1H, dd, *J*=10.8, 1.2 Hz, 17β-H), 5.15 (1H, m, 3-H), 5.92 (1H, dd, *J*=17.2, 10.8 Hz, 16-H), 6.19 1H, d, *J*=16.2 Hz, 7-H), 6.29 (1H, d, *J*=16.2 Hz, 8-H), 6.80 (2H, ABq, *J*=8.6 Hz, 11,13-H), 7.28 (2H, ABq, *J*=8.6 Hz, 10,14-H). ^{13}C-NMR ($CDCl_3$, δ): 17.6 (1-C), 23.2 (4-C), 23.3 (15-C), 25.7 (18-C), 41.2 (5-C), 42.5 (6-C), 111.9 (17-C), 115.4 (11,13-C), 124.8 (3-C), 126.5 (8-C), 127.3 (10,14-C), 130.8 (9-C), 131.2 (2-C), 135.8 (7-C), 145.9 (16-C), 154.6 (12-C).

Acetyl (+)-bakuchiol 2

(+)-Bakuchiol **1** was acetylated by the conventional manner, using the Ac_2O/pyridine to give a compound **2** acetyl-(+)-bakuchiol.

Colourless liquid. $[\alpha]_D=+36$ ($CHCl_3$, c=1.0) UV: $\lambda_{max}=255$ nm (MeOH), MS: *m/z* (rel. int.); 298 (M^+, 10), 256 (15), 174 (20), 173 (100), 158 (15), 145 (20), 107 (20). ^{1}H-NMR ($CDCl_3$, δ): 1.20 (3H, s, 15-CH_3), 1.50 (2H, m, 5-CH_2-), 1.58 (3H, s, 1-CH_3), 1.67 (3H, s, 18-CH_3), 1.94 (2H, m, 4-CH_2), 2.28 (3H, s, -$OCOCH_3$), 5.02 (1H, dd, *J*=17.2, 1.2 Hz, 17α-H), 5.03 (1H, dd, *J*=10.8, 1.2 Hz, 17β-H), 5.10 (1H, m, 3-H), 5.85 (1H, dd, *J*=17.2, 10.8 Hz, 16-H), 6.12 (1H, d, *J*=16.2 Hz, 7-H), 6.30 (1H, d, *J*=16.2 Hz, H-8), 7.01 (2H, ABq, *J*=8.6 Hz, 11,13-H), 7.37 (2H, ABq, *J*=8.6 Hz, 10,14-H).

(+)-bakuchiol 2,3-epoxide 3

(+)-Bakuchiol (**1**, *ca.* 40 mg) was dissolved in 1.0 m*l* of CH_2Cl_2 and stirred on an ice bath, in which added slowly the 30 mg of MCPBA (*m*-chloroperbenzoic acid) in 2 m*l* of CH_2Cl_2. After being stirred at RT, overnight, the reaction mixture was directly poured into the silica gel column and purified by chromatograpohy using 2% MeOH in CH_2Cl_2 as an eluent, which yield *ca* 15 mg of the (+)-baku-

chiol 2,3-epoxide **3**.

Colourless liquid, UV : λ_{max}=260 nm (MeOH), MS : *m/z* (rel. int.); 272 (M^+, 10), 174 (15), 173 (100), 158 (15), 145 (20), 107 (20). ^{1}H-NMR ($CDCl_3$, δ) : 1.12 (3H, s, 15-CH_3), 1.20 (3H, s, 1-CH_3), 1.25 (3H, s, 18-CH_3), 1.48 (2H, m, 5-CH_2-), 1.52 (2H, m, 4-CH_2), 2.69 (1H, m, 3-H), 4.96 (1H, dd, *J*=17.2, 1.2 Hz,, 17α-H), 5.00 (1H, dd, *J*=10.8, 1.2 Hz, 17β-H), 5.79 (1H, dd, *J*=17.2, 10.8 Hz, 16-H), 5.95 (1H, d, *J*=16.2 Hz, 7-H), 6.20 (1H, d, *J*=16.2 Hz, 8-H), 6.70 (2H, ABq, *J*=8.6 Hz, 11,13-H), 7.12 (2H, ABq, *J*=8.6 Hz, 10,14-H).

Compound 4

The acetyl (+)-bakuchiol **2**, *ca.* 50 mg was dissolved in 0.5 m*l* of AcOH, in which added 50 mg of CrO_3 in 2.5 m*l* of AcOH slowly. This reaction mixture was stirred for 3 hours at RT and then poured into 50 m*l* of water followed by extraction with EtOAc. The EtOAc layer was concentrated to dryness and purified by the silica gel column chromatography (CH_2Cl_2/MeOH=20/1), which was dissolved in 10 m*l* of 2.5% NH_3/MeOH and stirred for one hour at RT. The reaction mixture was evaporated to dryness, dissolved in CH_2Cl_2 and purified by the preparative TLC. (CH_2Cl_2/MeOH=20/1) to yield 8 mg of compound **4**.

Pale yellow liquid. UV : λ_{max}=260 nm (MeOH), MS : *m/z* (rel. int.); 246 (M^+, 20), 174 (15), 173 (100), 158 (14), 145 (24), 107 (15). ^{1}H-NMR ($CDCl_3$, δ) : 1.11 (3H, s, 15-CH_3), 1.75 (2H, m, 5-CH_2-), 2.27 (2H, m, 4-CH_2), 4.94 (1H, dd, *J*=17.2, 1.2 Hz, 17α-H), 4.98 (1H, dd, *J*=10.8, 1.2 Hz, 17β-H), 5.77 (1H, dd, *J*=17.2, 10.8 Hz, 16-H), 5.90 (1H, d, *J*=16.2 Hz, 7-H), 6.18 (1H, d, *J*=16.2 Hz, 8-H), 6.65 (2H, ABq, *J*=8.6 Hz, 11,13-H), 7.15 (2H, ABq, *J*=8.6 Hz, 10,14-H). ^{13}C-NMR ($CDCl_3$, δ) : 23.3 (15-C), 29.7 (5-C), 35.2 (4-C), 42.0 (6-C), 112.8 (17-C), 115.4 (11,13-C), 127.4 (10,14-C), 127.5 (8-C), 130.2 (9-C), 134.2 (7-C), 144.8 (16-C), 155.0 (12-C), 179.7 (COOH).

Compound 5

Compound **4** was converted to the methyl ester **5** by means of conventional methylation with the diazomethane in ether.

Pale yellow liquid. UV : λ_{max}=260 nm (MeOH), MS : *m/z* (rel. int.); 260 (M^+, 20), 174 (14), 173 (100), 158 (12), 145 (20), 107 (16). ^{1}H-NMR ($CDCl_3$, δ) : 1.11 (3H, s, 15-CH_3), 1.76 (2H, m, 5-CH_2-), 2.24 (2H, m, 4-CH_2), 3.56 (3H, s, $COOCH_3$), 4.94 (1H, dd, *J*=17.2, 1.2 Hz, 17α-H), 4.98 (1H, dd, *J*=10.8, 1.2 Hz, 17β-H), 5.77 (1H, dd, *J*=17.2, 10.8 Hz, 16-H), 5.92 (1H, d, *J*=16.2 Hz, 7-H), 6.18 (1H, d, *J*=16.2 Hz, 8-H), 6.65 (2H, ABq, *J*=8.6 Hz, 11,13-H), 7.15 (2H, ABq, *J*=8.6 Hz, 10,14-H).

Table I. Antitumoral activity of (+)-bakuchiol and its analogues 2-5 against human cancer cell lines (A549, SK-OV-3, SK-MEL-2, XF498 and HCT 15)

Comp./ cell	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	12*	15	10	12	12
2	>100	>100	>100	>100	>100
3	9	11	8	11	7
4	38	45	10	72	68
5	48	>100	14	45	50

*ED_{50} value of compound against each cancer cell line, which was defined as a concentration (μg/m*l*) that caused 50% inhibition of cell growth *in vitro*.

RESULTS AND DISCUSSION

The bioassay guided fractionation of *Psoralea corylifolia* (Leguminosae) led us the the isolation of a (+)-bakuchiol **1** as an active principle of the antitumoral or cytotoxic property *in vitro*. The chemical structure of the (+)-bakuchiol **1** was determined by the spectral analysis which was completely identical with reported data[4,5]. It was observed to exhibit a mild antitumoral activity against all five kinds of examined human tumor cells (Table I). Presumably, it seems that (+)-bakuchiol **1** showed a non-specific cytotoxic activity toward tumor cells.

The (+)-bukuchiol **1**, a major component of *Psoralea corylifolia* was firstly isolated from this plant by G. Mehta *et al.* and fully determined[4] including the absolute configuration at 6-positioned carbon as (S)-chirality[5] and even the total synthesis in 1973[6]. It has been noted that an oil obtainable from the seeds of *Psoralea corylifolia* by hexane extraction as well as the (+)-bakuchiol **1** itself inhibited the growth of *Staphylococcus aureus* (H.114)[7,8], and also that **1** showed the inhibition of mutagenic action of 2-aminoanthracene on *Salmonella typhimurium*(T-98)[9]. Recently, Iwamura *et al.* reported that **1** showed the cytotoxic activity against L929

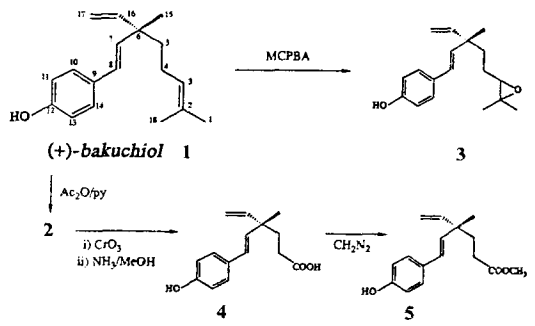

Fig. 1. The (+)-bakuchiol 1 and the synthetic scheme of its analogues 2-5.

(murine transformed fibroblast) cells in cell culture[10].

We had also examined the antitumoral activity of synthesized analogues **2-5** of **1** against each kind of human cancer cell for the purpose of investigating the active site of **1**. The antitumoral activity **1** and its analogues was summarized in Table I, and the synthetic scheme of analogues **2-5** in Fig. 1.

The (+)-bakuchiol **1** and (+)-bakuchiol 2,3-epoxide **3** showed a similar activity, whereas the others less activity. Although, it is not enough to tell the actual active site of (+)-bakuchiol **1**, this result suggested that the phenolic hydroxyl group is necessary for the antitumoral activity of **1**, and that the terminal isoprenyl moiety (C-1 to C-4 including C-18) also contributes to the activity. Anyhow, even if the antitumoral activity of (+)-bakuchiol **1** was not so excellent as that of other well-known anticancer agents (ED_{50}<4.0 μg/m*l in vitro*), and was not completely confirmed *in vivo* yet, it has some merits to become a promising lead for the development of new anticancer agent. The most big advantage of (+)-bakuchiol **1** is its high content in *Psoralea corylifolia* (more than 4%), so it could provide at least a good source for the development of new anticancer agent.

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