Arch. Pharm. Res. Vol.19, No.3, pp. 228-230, 1996

Synthesis and Antitumor Activity of Cannabigerol

Seung-Hwa Baek, Du Seok Han*, Chan Nam Yook, Young Chae Kim and Jung Suk Kwak**

**Department of Chemistry and Department of Oral Anatomy, Wonkwang University, Iksan 570-749, **Department of Health Hygienic, Wonkwang Public Health Junior College, Iksan 570-750, Korea*

(Received November 17, 1995)

Cannabigerol(3) was synthesized and evaluated for its inhibitory activity against mouse skin melanoma cells. Cannabigerol displayed significant antitumor activity [inhibitory concentration (IC_{50})=31.31 μg/mL] *in vitro* assay.

Key words : Cannabigerol, Antitumor activity, Mouse skin melanoma cell

INTRODUCTION

Cannabis has been used for therapeutic purposes for thousands years. It is fascinating that pharmacohistory is characterized by a progression from empirical use and anecdotal reports to the more critical scrutiny of modern scientific investigation. It has been known for its many therapeutic properties and has been widely used in various preparations in folklore medicine (Mechoulam, 1986). The cannabinoids, the active constituents of the plant, have numerous pharmacological properties including psychotropic effects, bronchodilation, increased heart rate, reduced intraocular pressure, analgesia, alteration of body temperature, anticonvulsant activity and others (Dewey, 1986).

Dronabinol, δ-9-tetrahydrocannabinol (δ-9-THC) in sesame oil, has been used for several years as an antiemetic for patients receiving cancer chemotherapy.

Plasse and his colleagues (1991) reported that dronabinol (THC in a capsule form) improved mood and appetite of patients with AIDS and cancer receiving chemo therapy. It has proven to be an extremely safe drug. While side effects are common, especially sedation and psychotropic symptoms, they are usually mild to moderate (Kleinman *et al.*, 1983; McCabe *et al.*, 1988; Sallan *et al.*, 1980). They resolve rapidly, usually within hours after discontinuation of therapy. Plasse *et al.* (1991) reported that dronabinol was not persistent or had fatal side effects. Colasanti *et al.* (1988) reported on the ability of topical δ-9-tetrahydrocannabinol (δ-9-THC) and cannabigerol (in a polyethylene glycol vehicle) to decrease intraocular pressure (IOP) in cats by an aqueous outflow mechanism. Topical cannabigerol appears effective and devoid of toxicity in cats, but its water insolubility (like δ-9-THC) may hinder formulation of a suitable topical dosage form for human use.

In the previous study (Baek *et al.*, 1985 and 1995), we reported a simple and convenient method for the intermolecular Friedel-Crafts alkylation reaction in the synthesis of cannabigerol type cannabinoids from geraniol and 5-alkylresorcinol in the presence of boron trifluoride-diethyl ether and silica in methylene chloride at room temperature for 2 days. The synthetic routes available are not of practical values as we lead to cannabigerol in mediocre yields.

Cannabigerol was prepared by using the standard synthetic route outlined in Fig. 1. Cannabigerol, a nonpsychoactive constituent, was identified by comparison of its spectral data (TLC, MS, NMR, UV and IR) with those published or by direct comparison (Baek *et al.*, 1985 and 1991). This compound was evaluated for the antitumor efficacy against mouse skin melanoma cells and showed a significant activity with IC_{50}=3.31 μg/ml. Geraniol (1) exhibits a little significant antitumor activity (Kang *et al.*, 1985; Baik *et*

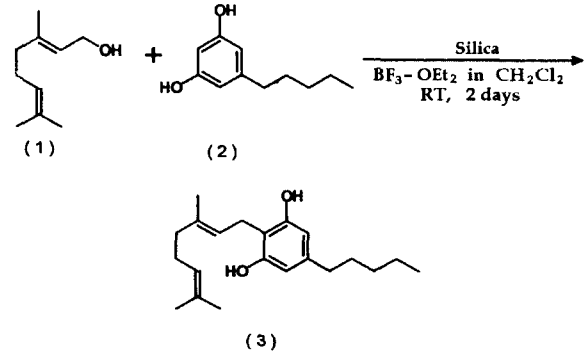

Fig. 1.

Correspondence to: Du Seok Han, Department of Chemistry and Department of Oral Anatomy, Wonkwang University, Iksan 570-749

al., 1988). Cannabigerol (3) showed a low IC_{50} value of 31.3l μg/mL. However, olivetol (2) is devoid of activity. Cannabigerol (3) has been selected as a lead compounds for further examinations.

In hopes of finding better therapeutic agents for antitumor activity, we report here the synthesis of cannabigerol and its antitumor activity against mouse skin melanoma cells.

MATERIALS AND METHODS

IR spectra were recorded on a Perkin-Elmer 457 grating infrared spectrophotometer. ^{1}H-NMR spectra were obtained on a Bruker WH-200 and WH-300 pulsed FT spectrometers. Chemical shifts are given in parts per million downfield from Me_4Si internal standard. Mass spectra were recorded on a Varian Mat CH-5 mass spectrometer. Analytical TLC was performed by using commercially available silica plates (Polygram sil N-HR/UV_{254}), and the plates were visualized with fast blue phenol reagent. Medium pressure liquid chromatography was performed on an ALTEX glass column, 1 meter long, diameter 9 mm internal using an FMI pump and silica gel 60 (230-400 mesh) purchased from Merck. Fractions were collected with LKB 2070 or LKB 7000 fraction collectors at a rate of 2-10 mL/min. Tumor cells for the experiments were obtained from Korean Cell Line Bank in the Seoul National University, which were currently used in the National Cancer Institute,s in vitro anticancer-drug screening. Tumor cell lines were grown in RPMI-1640 medium supple mented with 10% FBS, streptomycin 0.1% mg/mL and penicillin 100 units/mL at 37°C in 5% carbon dioxide, Cells were dissociated with 0. 25% trypsin just before transferring for experiment and were counted by Hemcytomer. Cytotoxic activities of cannabigerol against mouse skin melanoma cells were measured by the MTT method.

Preparation of Cannabigerol (3) (Baek *et al.*, 1985 and 1995).

BF_3-diethyl ether was added under nitrogen to a stirred suspension of silica (230-400 mesh ASTM) (2 g) in dichloromethane (20 mL). The mixture was stirred for 15 min at room temperature. Geraniol (1) (232 mg, 1.75 mmol) and olivetol (2) (180 mg, 1.0 mmol) in dichloromethane (5 mL) were added to the suspension by a syringe. The reaction mixture was stirred for 2 days, and was then worked up. The reaction product was separated on silica-gel column chromatography (10% ethyl acetate/petroleum ether) to give 93 mg (29% yield) of cannabigerol (3). Cannabigerol(3) thus obtained was identified by comparison of its spectral data (TLC, MS, NMR and IR) with those published or by direct comparison with an authentic sample.

Evaluation of antitumor activity

The antitumor activities of compounds 1-3 was determined by the modification of the literature methods (Mosmann, 1983; Carmichael *et al.*, 1987).

MTT-Microculture Tetrazolium Assay.

The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co. St. Louis, M. O.) by mitochondrial dehydrogenase of vial cells to a blue water-nonsoluble formazan crystal product which can be measured spectrophotometrically (Mosmann, 1983; Carmichael *et al.*, 1987). Following appropriate incubation of mouse skin melanoma cells in the presence or absence of cannabigerol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H- tetrazolium bromide (MTT) was added to each well and incubated at 37°C for a further 3hrs before processing as described below. Mouse skin melanoma cells were cultured in RPMI 1640 medium (Gubco Laboratories) containing 10% fetal bovine serum. 1×10^5 exponentially growing tumor cells were cultured for 48 hrs at 37°C in a humidified 5% CO_2 incubator. Well growing cells were harvested, counted and inoculated at the concentrations of 5×10^4 cells/mL into 24-well multidish plates. After 24 hrs, synthesized compounds were applied to triplicate culture wells and the cultures were incubated at 37°C for 24 hrs. Following this incubation, 20 μL of MTT solution (5 mg/mL in phosphate buffer solution; KCL 0.2 g, KH_2PO_4 0.2 g, NaCL 8.0 g, Na_2HPO_4 1.15 g, $MgCL_2$ 0. 101 g/L, pH=7.4) was added to microculture wells. After 3 hrs incubation at 37°C, the supernatant was removed from each well and 100 μL of 100% DMSO was added to solubilize the formazan crystals which were formed by the cellular reduction of MTT. After thorough mixing with a mechanical plate mixer, absorbance spectra were read on ELISA processor II Microplate Reader (Behring Co.) at a wavelength of 540 nm and a reference wavelength of 650 nm (absorbance peak for DMSO). All measurements were carried out in triplicates. There were good reproducibility between replicate wells with standard errors S +10%

ACKNOWLEDGEMENT

This work was supported by the Wonkwang University, 1995.

REFERENCES CITED

Baek, S. H., Yook, C. N., and Han, D. S.: Boron trifluoride etherate on alumina-A modified Lewis acid reagent(V). A convenient single step synthesis

of cannabinoids. *Bull. Kor. Chem. Soc.* 16(3), 293-296 (1991).

Baek, S. H., Srebnik, M., and Mechoulam, R.: Boron trifluoride etherate on alumina-A modified Lewis acid reagent. An improved synthesis of cannabidiol. *Tetrahedron Lett.* 26(8), 1083-1086 (1985).

Baik, K. U., and Ahn, B. Z.: Cyctotoxic activities of some geranylated flavones against L 1210 cell. *J. Pharm. Soc. Korea*, 32(2), 125-128 (1988).

Colasanti, B. K., and Gong, H. Marihuana 87, Chesher, G.; Consroe, P.; Musty, R. Ed., Australian Government Publishing Service, Canberra, pp.119-124 (1988).

Carmichael, J., Degraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B.: Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* 47, 936-942 (1987).

Dewey, W. L.: Cannabinoid pharmacology. *Pharmacol. Rev.* 38, 151-178 (1986).

Kleinman, S., Weitzman, S. A., Cassem, N., and Andrews, E.: Double blind trial of δ-9-tetrahydrocannabinol (THC) as an adjunct to prochlorperazine for chemotherapy-induced vomiting. *Curr. Ther. Res.* 34, 1014-1017 (1983).

Kang, K. S., Ryu, S. H., and Ahn, B. Z.: Antineoplastic natural products and the analogues VI. *Arch. Pharm. Res.* 8, 187-190 (1985).

Mechoulam, R.: In Cannabinoid as therapeutic agents, Boca Raton, CRC Press Inc., (1986).

Mosmann, T. J.: *Rapid colorimetric assays for cellular growth and survival : application to proliferation and cytotoxicity assays. Immunol. Methods*, 65, 55-63 (1983).

McCabe, M., Smith, F. P., Macdonald, J. S., and Woolley, P. V.: *Efficacy of tetrahydrocannabinol in patients refractory to standard antiemetic therapy. Invest. New Drugs.* 6, 243-246 (1988).

Plasse, T. F., Gorter, R. W., Krasnow, S. H., Lane, M., Shepard, K. V., and Wadleig H, R. G.: Recent clinical experince with dronabinol. *Pharmacol. Biochem. Behav.* 40, 695-700 (1991).

Sallan, S. E., Cronin, C., Zelen, M., and Zinberg, N. E.: Antiemetics in patients receiving chemotherapy for cancer: A randomized comparison of δ-9-tetrahydrocannabinol and prochlorperazine. *N. Engl. J. Med.* 302, 135-138 (1980)