Cytotoxicity of Green Tea Flavor Compounds against Two Solid Tumor Cells

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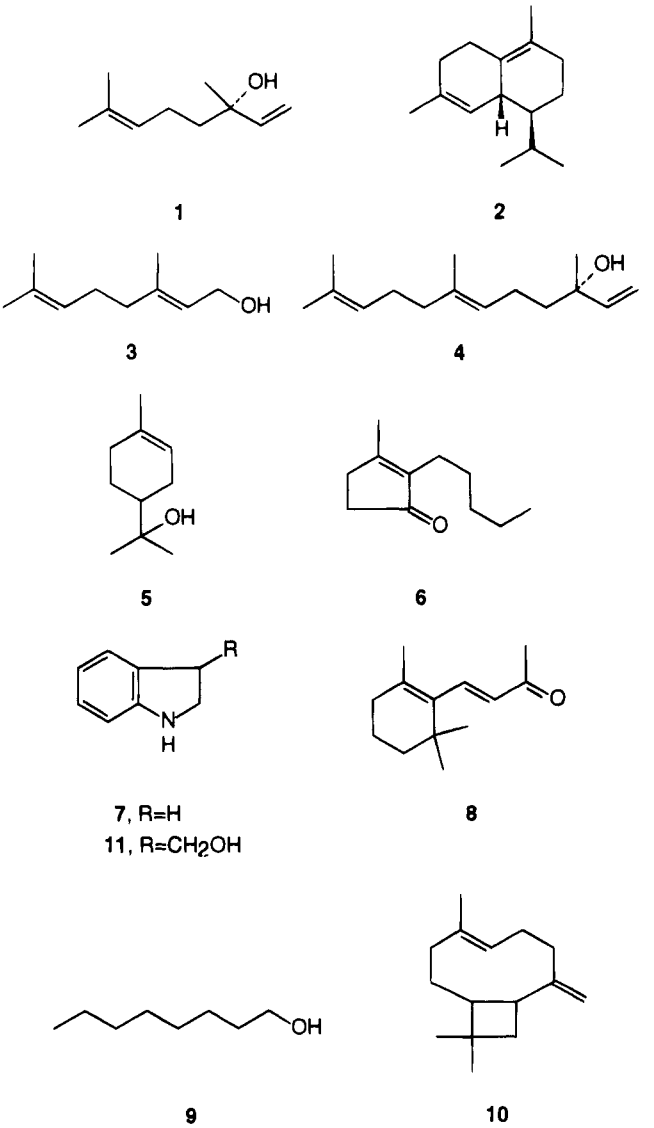
The cytotoxicity of the 10 most abundant compounds of green tea flavor was tested against two human carcinoma cell lines. Among these compounds, nerolidol, β-ionone, δ-cadinene, and β-caryophyllene were found to exhibit moderate *in vitro* cytotoxicity. These flavor compounds were also tested in combination with indole and indole-3-carbinol to enhance their activity.

Keywords: *Green tea; flavor compounds; cytotoxicity*

INTRODUCTION

We now control many tumors with available antitumor agents. However, the need for new antitumor agents is pressing, especially against solid tumors. The unacceptable and undesired side effects of many antitumor agents present a major problem that needs to be improved, especially since antitumor agents need to be used for long durations. It seems that edible plants, food spices, and daily beverages that have been continuously consumed by many people for many years may be a superior source of new antitumor agents (Kubo et al., 1993). In fact, a number of naturally occurring compounds from these sources have shown inhibitory activity in several types of tumorigenesis assays in animals (Lam and Hasegawa, 1989; Lam and Zheng, 1992; Wattenberg and Lam, 1984; Wattenberg, 1985).

Green tea is one of the most widely consumed beverages, especially in Japan. Recently, several polar polyphenolic compounds in green tea have been found to inhibit tumor promotion in mouse skin (Fujita et al., 1989; Yoshizawa et al., 1987). These polyphenolic compounds have also been reported to show anticarcinogenic (Katiyar et al., 1993; Huang et al., 1992) and antioxidative (Burdon, 1993) activity. The latter activity is also reported to prevent cancer since free radicals are in some way related to cancer (Floyd, 1980). The popularity of green tea is attributed to its pleasant flavor, combined with its stimulating effects. Despite its popularity, the antitumor activity of nonpolar substances, particularly volatile flavor compounds, has not yet been investigated. Hence, we have examined the cytotoxicity of the 10 major green tea flavor compounds against two human carcinoma cell lines. This study was based largely on previous studies that indicated essential oils are a potential source of natural inhibitors of carcinogenesis (Lam and Zheng, 1991). The active principles from a regularly imbibed beverage such as green tea may be superior antitumor agents as compared to many non-natural products.



MATERIALS AND METHODS

Chemicals. The 10 flavor compounds (**1**–**10**) used for the assay were from our previous study (Kubo et al., 1992). For cytotoxic assay experiments, all chemicals were first dissolved in dimethyl sulfoxide (DMSO) that was purchased from Sigma Chemical Co. (St. Louis, MO). Indole-3-carbinol, formazan dye, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were also purchased from Sigma.

Cell Lines and Media. The cell lines BT-20 ATCC HTB 19, breast carcinoma isolated from human, and HeLa ATCC CCL 219, epithelioid cervix carcinoma cell from human, used for the assay were purchased from American Type Culture Collection (Rockville, MD). The selection of the cell lines was based on our continuing study of prostaglandin (PG) synthetase inhibitors from tropical plants (Kubo et al., 1987). Thus, breast carcinoma cells produce PGE_2 as they grow. Both cell lines were maintained on minimum essential medium

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Table 1. Cytotoxicity of the 10 Major Green Tea Flavor Compounds against BT-20 Breast and HeLa Epithelioid Cervix Carcinoma Cells

compound tested	IC_{50} (μg/mL) BT-20	HeLa
linalool (**1**)	>40	24.86
δ-cadinene (**2**)	3.86	3.66
geraniol (**3**)	18.02	22.09
nerolidol (**4**)	2.96	3.02
α-terpineol (**5**)	>40	>40
cis-jasmone (**6**)	>40	23.01
indole (**7**)	>40	>40
β-ionone (**8**)	3.23	2.96
1-octanol (**9**)	23.73	25.05
β-caryophyllene (**10**)	3.86	3.92
indole-3-carbinol (**11**)	>40	>40

(Eagle) with nonessential amino acids, supplemented with 10% (v/v) heat-inactivated fetal calf serum, and incubated in a humidified atmosphere of 5% CO_2–95% air at 37 °C. The human monolayer cultures were subcultured with a 0.25% trypsin/EDTA solution.

Cytotoxic Assay. The cytotoxic assays were performed according to a microculture tetrazolium (MTT) method (Carmichael et al., 1987). Briefly, cells were harvested and inoculated into 96-well microtiter plates at 4000–6000 cells/well, with various concentrations of the samples prepared by serial 2-fold dilutions. After incubation for 72 h, 50 μg/mL MTT (3 mg/mL in phosphate-buffered saline, pH 7.2) was added. The formazan dye was solubilized by adding 100 μg/mL DMSO to each well, followed by gentle shaking. The extinction coefficient was measured for each well using a Uniskan I Photometer Labsystem (Labsystems, Helsinki, Finland), at a wavelength of 620 nm.

Combination Study. The combination data were obtained according to the checkerboard method (Norden et al., 1979). Thus, a series of the 2-fold dilutions of indole or indole-3-carbinol was tested in combination with 2-fold dilutions of the other. Each compound was tested at least twice.

RESULTS AND DISCUSSION

In a previous paper (Kubo et al., 1992), we reported the antimicrobial activity of the 10 most abundant volatile flavor compounds identified in green tea, namely linalool (**1**), δ-cadinene (**2**), geraniol (**3**), nerolidol (**4**), α-terpineol (**5**), *cis*-jasmone (**6**), indole (**7**), β-ionone (**8**), 1-octanol (**9**), and β-caryophyllene (**10**), in decreasing concentration. These volatile compounds were selected from the list reported previously (Nose et al., 1971) since the complex green tea flavor contains over 100 volatile compounds (Flament, 1991; Yamaguchi and Shibamoto, 1981). Most of the compounds selected are classified as Generally Recognized As Safe (GRAS).

The cytotoxicity of the same flavor compounds (**1**–**10**) was first tested against BT-20 breast carcinoma cells. In the preliminary assay, only nerolidol, β-ionone, δ-cadinene, and β-caryophyllene exhibited significant (<4 μg/mL) *in vitro* cytotoxicity. Nevertheless, the 50% inhibitory concentration (IC_{50}) values of these 10 volatile compounds were obtained. The highest concentration of an individual compound tested was 40 μg/mL because of their solubility limitation in the water-based test solution. The results are given in Table 1. Among them, the most effective cytotoxicity was observed for nerolidol (**4**) with an IC_{50} = 2.96 μg/mL, followed by β-ionone (**8**) with an IC_{50} = 3.23 μg/mL, and then δ-cadinene (**2**) and β-caryophyllene (**10**) with IC_{50} = 3.86 μg/mL for each. Interestingly, geraniol (**3**) and 1-octanol (**9**) also demonstrated weak (>10 μg/mL) activity.

In addition, the same 10 volatile compounds were assayed against HeLa epithelioid cervix carcinoma cells. The above-mentioned four cytotoxic compounds against BT-20 breast carcinoma cells also exhibited significant (<4 μg/mL) activity against HeLa cells. In the case against this human carcinoma cells, the most potent cytotoxicity was observed for β-ionone (**8**) with an IC_{50} = 2.96 μg/mL, followed by nerolidol (**4**) with an IC_{50} = 3.02 μg/mL, and then δ-cadinene (**2**) with an IC_{50} = 3.66 μg/mL. The IC_{50} of β-caryophyllene (**10**) was found to be 3.92 μg/mL. In addition, linalool (**1**), geraniol (**3**), *cis*-jasmone (**6**), and 1-octanol (**9**) also demonstrated weak (>10 μg/mL) activity against HeLa epithelioid cervix cells. The IC_{50} values of the 10 flavor compounds against this cell line are also presented in Table 1.

The cytotoxicity of these volatiles may not be potent enough to use them as leading compounds for drug design, but they are worthy of further investigation as natural products isolated from a daily beverage. Importantly, these volatile compounds are identified in many edible plants, food spices, and beverages and are frequently used for fragrance and flavor (Bauer et al., 1991). There is no doubt that these volatile compounds have long been consumed by many people. For example, the most potent cytotoxic volatile in green tea flavor, nerolidol (**4**), is also a common component of many essential oils. The most abundant volatile in green tea flavor, linalool (**1**), is also found in many food spices such as coriander, lavender, sage, and thyme (Maarse, 1991), often as the main component. The IC_{50} of 24.86 μg/mL against HeLa cells of this monoterpene alcohol is not as potent, but the amount of its daily intake can be high. This may also be true with other volatile compounds that exhibit related cancer control activities. This kind of information has just come into the light. For example, in this experiment geraniol (**3**) exhibited only weak cytotoxicity with an IC_{50} of 18.02 and 22.09 μg/mL against BT-20 and HeLa carcinoma cells, respectively, but it was recently reported to show high glutathione *S*-transferase-inducing activity (Zheng et al., 1993), which is believed to be a mechanism in carcinogen detoxification (Chasseaud, 1979). The same glutathione *S*-transferase-inducing activity has also been reported with β-caryophyllene (**10**) (Zheng et al., 1992).

Anzai et al. demonstrated that 2′-deoxy-5-azacytidine can synergistically interact with topotecan against human tumor cell lines (Anzai et al., 1992). This interaction can translate into an improved antitumor effect and may delay the development of drug resistance. In our previous study of antimicrobial activity of the same green tea flavor compounds, indole (**7**) was found to enhance the antibacterial activity of δ-cadinene (**2**) 128-fold against one of the most important cariogenic bacteria, *Streptococcus mutans* (Kubo et al., 1992; Muroi and Kubo, 1993). Thus, its minimum inhibitory concentration (MIC) was lowered from 800 to 6.25 μg/mL by combining it with a sublethal amount of indole. Hence, δ-cadinene as well as β-ionone and nerolidol was examined in combination with indole against BT-20 breast carcinoma cells. In addition to indole, indole-3-carbinol (**11**) isolated from cruciferous vegetables was also tested in combination with these compounds since indoles have been shown to inhibit chemically induced tumorigenesis in animal models (Loub et al., 1975). However, in contrast to the antibacterial activity, neither indole nor indole-3-carbinol dramatically increased their cytotoxicity, although a more appropriate experiment may be needed (Frost et al., 1990). The combination effect was only additive.

Although the amount of the volatile flavor compounds in green tea was reported to be extremely small (Nose et al., 1971), it could be concluded that continuous green tea consumption may be effective for controlling tumors because of the combination of cytotoxic, tumor promotion inhibition, antioxidative, and glutathione *S*-transferase-inducing effects. More importantly, the above-mentioned cytotoxic and glutathione *S*-transferase-inducing volatile compounds are found in many edible plants, food spices, and daily beverages (Bauer et al., 1991). For example, β-caryophyllene (**10**) is approved by the U.S. Food and Drug Administration for food use (21 CFR 121.1164). Therefore, eating a well-balanced diet including vegetables and fruits, which contain large amounts of these volatile compounds, may help prevent cancer. In addition, these rather common volatile cytotoxic agents could have potential as dietary supplements.

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