Suppression of the Furylfuramide-Induced SOS Response by Monoterpenoids with a *p*-Menthane Skeleton Using the *Salmonella typhimurium* TA1535/pSK1002 *Umu* Test

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Suppression of the furylfuramide-induced SOS response by 25 kinds monoterpenoids (hydrocarbons, alcohols, ketones, and aldehydes) with a *p*-menthane skeleton was studied. Suppression of the SOS-inducing activity by monoterpenoids was determined in the *umu* test using *Salmonella typhimurium* TA1535/pSK1002. The terpene alcohols, ketones, and aldehydes had potent suppressive effects, but the hydrocarbons did not. Especially, (+)-menthol, (+)-pulegone, piperitenone, and cuminaldehyde were shown to have the most potent suppressive effects, and the ID_{50} (dose for 50% inhibition) was 0.52 μmol/mL.

Keywords: *Monoterpenoids; SOS response; umu test; Salmonella thypimurium TA1535/pSK1002; furylfuramide (AF-2)*

INTRODUCTION

Several short-term tests for screening environmental mutagens and carcinogens have been developed and are widely used in many laboratories (Ames et al., 1975; Kada et al., 1981). The Ames test is a convenient method for evaluating mutagenic activity (Ames et al., 1975), and several pieces of evidence have suggested that the mutagenic activity of a number of chemicals can be correlated well with the carcinogenic activity so far reported (McCann et al., 1975; Shugimura et al., 1976).

The SOS response appears to be induced by an alteration in DNA synthesis, either directly by DNA damage blocking to the replication fork or indirectly by antibiotics, such as novobiocin, that inhibit DNA synthesis. The *umu* test system was developed to evaluate the genotoxic activity of a wide variety of environmental carcinogens and mutagens, using the expression of one of the SOS genes to detect DNA-damaging agents (Oda et al., 1985; Nakamura et al., 1987). The results of this test are in agreement with the results of the Ames test and may be more useful with respect to simplicity, sensitivity, and rapidity (Reifferscheid et al., 1996).

Terpenoids are contained in essential oils from plants. Monoterpenoids have been isolated from the fragrant oils of many higher plants and are important in the perfumery and flavor industries. Monoterpenoids are also found in many marine organisms, where they are generally halogenated, and as insect pheromones and defense secretions. The biosynthetic pathways of the main classes of monoterpenes have been well studied. The menthane group comprises three isomeric types, *o*-, *m*-, and *p*-menthane. The *p*-menthanes are the most widespread and arise by a cyclization of a regular acyclic monoterpenoid. The *o*- and *m*-menthanes are much rarer and presumably arise by alkyl migration of *p*-menthanes.

As part of our continuing program to discover bioactive natural compounds, we have investigated antimutagenic compounds and suppressive compounds of SOS response in plants (Miyazawa et al., 1997, 1998a,b). Monoterpenoids with a *p*-menthane skeleton (Figrue 1) are contained in many kinds of mint oil, which is used as a flavor. Recently, some new bioactivities of *p*-menthanes have been reported (Crowell et al., 1992; Kubo et al., 1994). We have reported the inhibition of acetylcholinesterase activity by monoterpenoids (Miyazawa et al., 1997). However, there are no reports about the suppressive effect on chemical mutagen-induced SOS response by terpenoids. Therefore, in this study, we report the suppressive effect of 25 kinds of monoterpenoids with the *p*-menthane skeleton against chemical mutagen-induced SOS response. In addition, the structure−activity relationship of *p*-menthanes is also discussed.

EXPERIMENTAL PROCEDURES

Materials. Monoterpenoids [*p*-menthane skeleton (e.g., >97%)] were purchased from Taiyo Perfume Co., Ltd. (Osaka, Japan). Furylfuramide was purchased from Wako Pure Chemicals Co. (Osaka, Japan).

***Umu* Test.** The *umu* test for detecting the SOS-inducing activity of chemicals was carried out according to the method of Oda et al. (1985) using *S. typhimurium* TA1535/pSK1002, which has a plasmid pSK1002 that carries a *umuC′-′lacZ* fused gene. The level of β-galactosidase activity was measured by using a slight modification of Miller's method (Miller, 1972).

RESULTS AND DISCUSSION

The suppressive effects of compounds **1**−**25** were determined in the *umu* test. These results are shown

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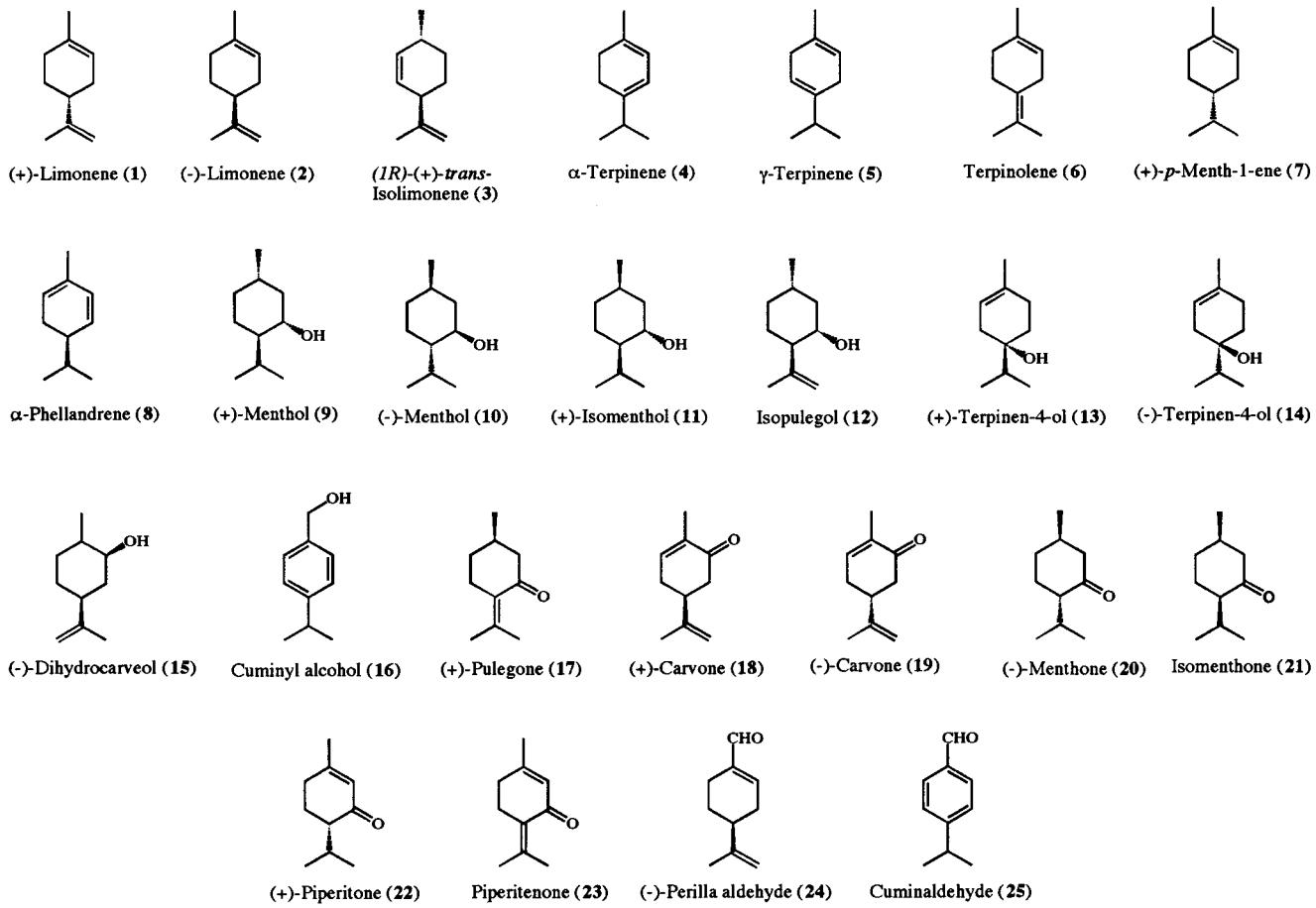

Figure 1. Structures of monoterpenoids with a *p*-menthane skeleton.

Table 1. Suppression of Furylfuramide[a]-Induced SOS Response by Monoterpenoids with a *p*-Menthane Skeleton

	compd	suppressive effect (1.0 μmol/mL[b]) (%)	ID_{50}[c] (μmol/mL)
1	(+)-limonene	8.2	
2	(−)-limonene	0	
3	1*R*-(+)-*trans*-isolimonene	4.1	
4	α-terpinene	2.8	
5	γ-terpinene	14.6	
6	terpinolene	2.5	
7	(+)-*p*-menth-1-ene	20.2	
8	α-phellandrene	6.3	
9	(+)-menthol	77.2	0.52
10	(−)-menthol	79.5	0.65
11	(+)-isomenthol	70.0	0.60
12	isopulegol	55.9	0.80
13	(+)-terpinen-4-ol	41.2	
14	(−)-terpinen-4-ol	57.3	0.92
15	dihydrocarvenol	61.5	0.85
16	cuminyl alcohol	29.3	
17	(+)-pulegone	67.4	0.52
18	(+)-carvone	51.8	0.90
19	(−)-carvone	44.6	
20	(−)-menthone	61.1	0.79
21	(−)-isomenthone	61.3	0.80
22	(+)-piperitone	21.7	
23	piperitenone	79.6	0.52
24	(−)-perilla aldehyde	70.3	0.60
25	cuminaldehyde	75.4	0.52

[a] Furylfuramide (1.0 μg/mL in DMSO) was added at 50 μL. Positive control was added at furylfuramide (0.05 mg) without terpenoids. [b] Hydrocarbons were added at a concentration of 0.07 μmol/mL. [c] Dose for 50% inhibition.

in Table 1 and Figure 2. Monoterpene hydrocarbons showed toxicity for *S. typhimurium* TA1535/pSK1002 at >0.07 μmol/mL. On the other hand, monoterpene alcohols, ketones, and aldehydes showed toxicity for this strain at >1.0 μmol/mL. To obtain dose−response data, hydrocarbons were evaluated at dose levels of 0.07, 0.035, and 0.015 μmol/mL. Alcohols, ketones, and aldehydes were evaluated at dose levels of 1.0, 0.6, 0.2, and 0.1 μmol/mL.

Suppressive Effects of Monoterpene Hydrocarbons. Monoterpene hydrocarbons have potent toxicity for *S. typhimurium* TA1535/pSK1002 at >0.07 μmol/mL. As shown in Figure 2a, γ-terpinene (**5**) and (+)-*p*-menth-1-ene (**7**) suppressed 14.6 and 20.2% of the furylfuramide-induced SOS response at a concentration of 0.07 μmol/mL. Other hydrocarbons showed weak or no suppressive effect against furylfuramide-induced SOS response.

Suppressive Effects of Alcohols. As shown in Figure 2b, monoterpene alcohol showed potent suppressive effects. Especially, (+)-menthol (**9**), (−)-menthol (**10**), (−)-isomenthol (**11**), and dihydrocarvenol (**15**) suppressed 77.2, 79.5, 70.0, and 61.5% of the furylfuramide-induced SOS response at a concentration of 1.0 μmol/mL, respectively. The ID_{50} values (50% inhibition dose) of compounds **9**, **10**, **11**, and **15** were 0.52, 0.65, 0.60, and 0.85 μmol/mL, respectively, but suppressive effects of (+)-terpinen-4-ol (**13**) and cuminyl alcohol (**16**) were weak.

Suppressive Effects of Ketones and Aldehydes. As shown Figure 2c, (+)-pulegone (**17**), (−)-menthon (**20**), (−)-isomenthon (**21**), piperitenone (**23**), (−)-perilla aldehyde (**24**), and cuminaldehyde (**25**) suppressed 67.4, 61.1, 61.3, 79.6, 70.3, and 75.4% of furylfuramide-induced SOS response at a concentration of 1.0 μmol/mL, respectively. The ID_{50} values of compounds **17**, **20**,

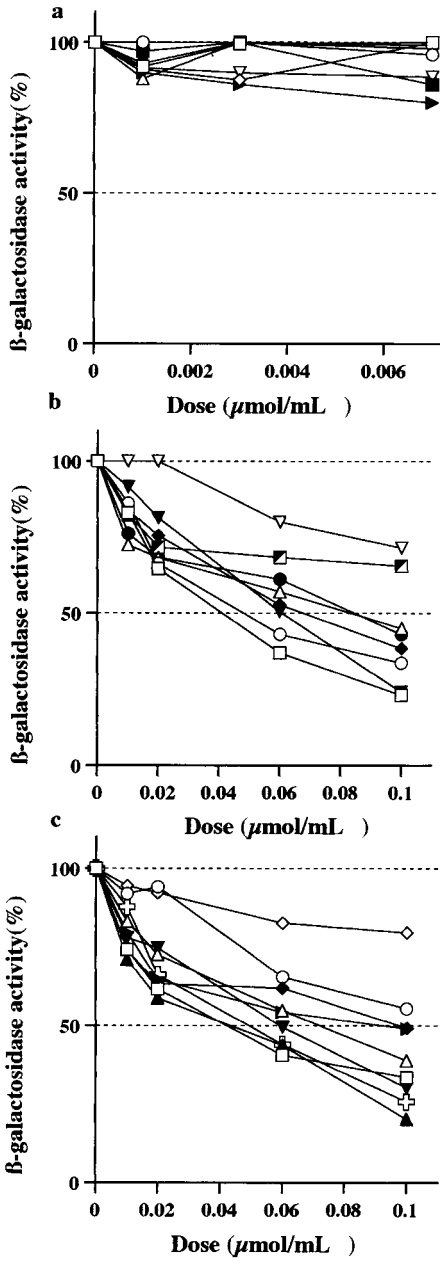

Figure 2. Suppressive effects of compounds **1**−**25** against the furylfuramide-induced SOS response in *S. typhimurium* TA1535/pSK1002: (a) hydrocarbons [(□) (+)-limonene (**1**); (◇) (−)-limonene (**2**); (○) (1*R*)-(+)-*trans*-isolimonene (**3**); (△) α-terpinene (**4**); (■) γ-terpinene (**5**); (●) terpinolene (**6**); (solid triangle pointing right) (+)-*p*-menth-1-ene (**7**); (▽) α-phellandrene (**8**)]; (b) alcohols [(□) (+)-menthol (**9**); (▼) (−)-menthol (**10**); (○) (+)-isomenthol (**11**); (△) isopulegol (**12**); (◩) (+)-terpinen-4-ol (**13**); (●) (−)-terpinen-4-ol (**14**); (◆) (−)-dyhydrocarvenol (**15**); (▽) cuminyl alcohol (**16**)]; (c) ketones and aldehydes [(□) (+)-pulegoe (**17**); (◆) (+)-carvone (**18**); (○) (−)-carvone (**19**); (△) (−)-menthone (**20**); (solid triangle pointing right) isomenthone (**21**); (◇) (+)-piperitone (**22**); (▲) piperitenone (**23**); (▼) (−)-perilla aldehyde (**24**); (⊕) cuminaldehyde (**25**)].

21, **23**, **24**, and **25** were 0.52, 0.79, 0.80, 0.52, 0.60, and 0.52 μmol/mL, respectively.

Relationship of the Structure and Activity of Monoterpenoids with *p*-Menthane Skeleton. As shown in Table 1 and Figure 2, monoterpene alcohols, ketones, and aldehydes showed potent suppressive effect against furylfuramide-induced SOS response. Especially, compounds **9**, **10**, **11**, **17**, **23**, **24**, and **25** showed greater suppressive effect than other compounds. However, monoterpene hydrocarbons, which had toxicity against *S. typhimurium* TA1535/pSK1002, did not show a suppressive effect. In alcohols, compounds **9**, **10**, **11**, **12**, and **15** had a greater suppressive effect than compounds **13**, **14**, and **16**. Also, compounds **17**, **20**, **21**, and **23** were more potent inhibitors of SOS response in ketones. The saturated six-membered ring is related to the strength of suppression of furylfuramide-induced SOS response. The inhibition of compound **9** was stronger than that of compound **12**. Compound **15** had a low suppressive effect, too. This result indicated that the presence of an isopropyl group decreases the strength of the inhibitory effect. In other hands, two aldehydes, nevertheless with unsaturated six-membered rings, showed potent inhibition. Thus, the aldehyde group is an important factor in the suppression of SOS-inducing activity.

There are several reports of antimutagenicity studies on the terpenoid (Ragasa et al., 1997a,b; Kuroda et al., 1987; Kakinuma et al., 1987). We have reported antimutagenic activity and suppressive effects of SOS response of a sesquiterpenoid (β-eudesmol). β-Eudesmol showed a suppressive effect of furylfuramide- and Trp-P-1-induced SOS response, which suggested that a hydroxy group at C-11 is a necessary factor to show activity. The effects of (+)-limonene and (+)-carvone on the chemically induced formation of tumors in mice and rats have been extensively studied by Wattenberg et al. (1989) and Gould et al. (1994). However, there are no reports of terpenoid suppression of SOS-inducing activity. In this paper, we reported the suppressive effect of monoterpenoids with *p*-menthane skeletons on furylfuramide-induced SOS response and suggested that alcohols, ketones, and aldehydes have potent suppressive effects in monoterpenoids. As to the important factor of inhibition by these compounds, two possibilities, the presence of a saturated six-membered ring and an aldehyde group, were anticipated. Plant terpenes may be available as SOS response inhibitors.

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