Effects of Carvone Compounds on Glutathione S-Transferase Activity in A/J Mice

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The monoterpene *d*-carvone is present in a large number of commonly used essential oils. Recently, it has been found to induce the activity of detoxifying enzymes and inhibit nitrosamine-induced carcinogenesis. To explore the structure–activity relationship, a series of carvone compounds were tested for their ability to induce increased activity of glutathione S-transferase in several tissues of A/J mice. The parent compound *d*-carvone exhibited the highest activity as an inducer in all of the tissues. The *α,β*-unsaturated ketone system in *d*-carvone appeared to be critical for the high enzyme-inducing activity, which proved the hypothesis that many anticarcinogenic enzyme inducers are Michael reaction acceptors containing olefinic bonds conjugated with electron-withdrawing groups. The effects of the carvone compounds on the tissue glutathione level were also determined. Several compounds were found to elevate GSH level significantly in the mouse forestomach, while *d*-carvone decreased GSH level in most of the tissues. Since the anticarcinogenic activity has been found to correlate with the ability to induce increased activity of detoxifying enzymes, carvone compounds can be a class of potential chemopreventive agents.

Dietary components such as cruciferous vegetables and their constituents have been shown to exert protective effects against the induction of cancer by chemical carcinogens (Committee on Diet, Nutrition, and Cancer, 1982). The isolation of inhibitors of carcinogenesis from edible plants offers a high potential for obtaining chemopreventive agents that can be useful in reducing the incidence of human cancer. The various types of dietary inhibitors of carcinogenesis and their mechanisms of action have been reviewed by Wattenberg (1985). Most of the anticarcinogenic dietary components isolated from plants are naturally occurring secondary metabolites (Loub et al., 1975; Wattenberg et al., 1976; Pantuck et al., 1976) including indoles (Loub et al., 1975), cafestol and kahweol (Wattenberg and Lam, 1984), limonin and nomilin (Lam and Hasegawa, 1989), and sulfur-containing compounds from onion and garlic oils (Belman, 1983; Wargovich, 1987). One group of anticarcinogenic natural products is essential oils, which consist of mainly volatile monoterpenes (Lam and Zheng, 1991a). A few of the monoterpenes are commonly present in a large number of essential oils and appear to be effective inhibitors of carcinogenesis. Carvone (1), which is present in caraway oil, dillweed oil, lemon oil, orange oil, mandarin peel oil, spearmint oil, kuromoji oil, and gingergrass oil (Formacek and Kubeczka, 1982; Merck & Co., Inc., 1989), was found to be both an inducer of detoxifying enzymes (Lam and Zheng, 1991a) and an inhibitor of nitrosamine-induced carcinogenesis in mice (Wattenberg et al., 1989).

One of the well-studied enzyme systems commonly induced by anticarcinogenic agents is glutathione S-transferase (GST) (Shankel et al., 1986; Chasseaud, 1979; Jakoby and Habig, 1980). GST has been investigated extensively as a major detoxifying enzyme system that catalyzes the conjugation of a wide variety of electrophiles with glutathione (GSH) to form less toxic, water-soluble substances that can be readily excreted. Since the reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, induction of GST activity by anticarcinogenic compounds is believed to be a major mechanism for carcinogen detoxification. The GST inducers are of particular interest because they have the capacity to inhibit

a wide range of carcinogens. Research in our laboratories has demonstrated that certain chemicals such as *d*-carvone (Lam and Zheng, 1991a), *d*-limonoids (Lam and Hasegawa, 1989), 2-*n*-heptylfuran, and 2-*n*-butylthiophene (Lam and Zheng, 1991b) that induce GST activity in a tissue can inhibit polycyclic aromatic hydrocarbon-induced neoplasia in that tissue. Other researchers have also shown a positive correlation between inhibition of tumorigenesis and enhancement of GST activity in the same tissues of mice by several classes of inhibitors (Sparnins and Wattenberg, 1981; Sparnins et al., 1982; Lam et al., 1982; Benson et al., 1978). Thus, the ability to enhance GST activity can be used for the screening and evaluation of potential inhibitors of chemical carcinogenesis. Recently, using the in vivo GST enzyme assay, we have determined the GST inducing potential of a large number of common essential oils and their byproducts (Lam and Zheng, 1991a). Some common constituents of essential oils such as *d*-carvone were found to induce GST activity in the target tissues of mice. At 20-mg dose the induction of GST by *d*-carvone in the forestomach, liver, colon, and small bowel was 3.68, 2.34, 2.63, and 2.30 times the control, respectively. If correlation of tumor inhibition and GST induction holds for these compounds, they can be effective chemopreventive agents.

L-γ-Glutamyl-L-cysteinylglycine or glutathione is a sulfhydryl-containing tripeptide that is the major constituent of the intracellular nonprotein sulfhydryl pool (Meister and Anderson, 1983). This important reducing agent and antioxidant is involved in maintaining cellular oxidation–reduction balance and protecting cells against free-radical species, reactive oxygen intermediates, and toxic xenobiotics. Topical application of GSH has been found to inhibit tumor progression in the skin model (Rotstein and Slaga, 1988). An increase of GSH level, therefore, is considered an important event in protection against carcinogens. Many of the inducers of GST have been found to induce increased level of GSH in various tissues. Elevation of GST activity concomitant with an increase in the GSH level forms a powerful detoxifying response against xenobiotics. For this reason, we have included

the determination of the GSH level as a screening assay for inhibitors of carcinogenesis.

In this study, a series of carvone compounds were selected to test for their effects on induction of GST in mice to establish structure–activity relationship (SAR) for the further development of more potent and selective analogues through structural modification. Carvone is a possible metabolite of limonene, which structurally differs from limonene by only a carbonyl group. However, *d*-carvone is much more active than *d*-limonene in the induction of increased GST activity (Lam and Zheng, 1991a). With the above observation, we would like to proceed with the SAR study by investigating the basic structure requirements for the induction of GST activity and the significance of the α,β-unsaturated ketone system of the carvone compounds. Since many inducers are also substrates for GST (Talalay et al., 1988), this SAR study should provide mechanistic information for the induction of detoxifying enzymes by carvone compounds and lead to the prediction of the structures of inducers with potential anticarcinogenic activity.

MATERIALS AND METHODS

General Procedure. Ultraviolet (UV) spectra were determined on a Beckman DU-65 spectrophotometer. Low- and high-resolution mass spectra (MS) were obtained by use of Kratos MS-25 and AEI MS-30 mass spectrometers, respectively. Nuclear magnetic resonance (NMR) spectra were recorded on a Nicolet NT-300 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H NMR spectra measured in CDCl$_3$. CDCl$_3$ (δ 77.00) was used as the internal reference for ^{13}C NMR spectra. The signal multiplicities were described using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Chemicals. Glutathione was purchased from Sigma Chemical Co. (St. Louis, MO). 1-Chloro-2,4-dinitrobenzene was obtained from Aldrich Chemical Co. (Milwaukee, WI). The following test compounds were of commercial origin (Aldrich) and were used without further purification: *d*-carvone (1), carvone hydrate or 5-(1-hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one (3), carvone acetate or 5-(1-acetoxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one (4), dihydrocarvone (5), carveol (7), carvyl acetate (8), dihydrocarveol (9), dihydrocarvyl acetate (10), and *trans*-sobrerol or *trans*-*p*-menth-6-ene-2,8-diol (11). All other chemicals were of reagent grade or purer and purchased from Aldrich unless noted otherwise. Carvotanacetone (2) and carvomenthone (6) were synthesized by catalytic hydrogenation of carvones as described below.

Carvotanacetone (2). Carvone (2, 3.0 g, 20 mmol) dissolved in methanol (12 mL) was hydrogenated in the presence of 5% palladium on calcium carbonate poisoned with lead (0.5 g) at room temperature and normal pressure. More catalyst was added at the end of the overnight reaction, which was monitored by thin-layer chromatography. The hydrogenation period was extended to 48 h to bring the reaction to completion. The catalyst was removed by filtration through a Celite pad and washed with methanol. The combined filtrate and washings were evaporated, and the crude product was purified by preparative TLC (silica gel GF plates purchased from Analtech: 2-mm thickness, 20 × 20 cm; solvent, hexane–ethyl acetate 95:5) to give compound 2 as a colorless oil (2.2 g, 14.5 mmol, 73% yield): high-resolution EI-MS calcd for C$_{10}$H$_{16}$O (M$^+$) 152.1201, obsvd 152.1189; UV (MeOH) λ_{max} (log ϵ) 243 (3.45) nm; ^{1}H NMR (CDCl$_3$) δ 0.92 (d, 6 H, J = 6.6, Hz, 9-CH$_3$ and 10-CH$_3$), 1.58 (m, 1 H, H-8), 1.77 (s, 3 H, 7-CH$_3$), 1.84 (m, 1 H, H-5), 2.02–2.58 (m, 4 H, 4-CH$_2$ and 6-CH$_2$), 6.74 (m, 1 H, H-3); ^{13}C NMR (CDCl$_3$) δ 15.59 (C-7), 19.45 (C-9), 19.91 (C-10), 29.79 (C-8), 31.94 (C-4), 41.91 (C-5), 44.18 (C-6), 135.18 (C-2), 145.42 (C-3), 200.76 (C-1); EI-MS m/z (relative intensity) 152 (M$^+$, 68), 124 (M$^+$ – CO, 10), 109 (M$^+$ – C$_3$H$_7$, 40), 95 (M$^+$ – C$_4$H$_9$, 27), 82 (M$^+$ – C$_5$H$_{10}$, part A from RDA cleavage, 100), 81 (M$^+$ – C$_3$H$_7$ – CO, 67), 70 (M$^+$ – C$_5$H$_6$O, part B from RDA cleavage, 13), 69 (C$_5$H$_9^+$, 22), 55 (C$_4$H$_7^+$, 17), 54 (M$^+$ – C$_5$H$_{10}$ – CO, 17), 43 (C$_3$H$_7^+$, 32), 41 (C$_3$H$_5^+$, 26).
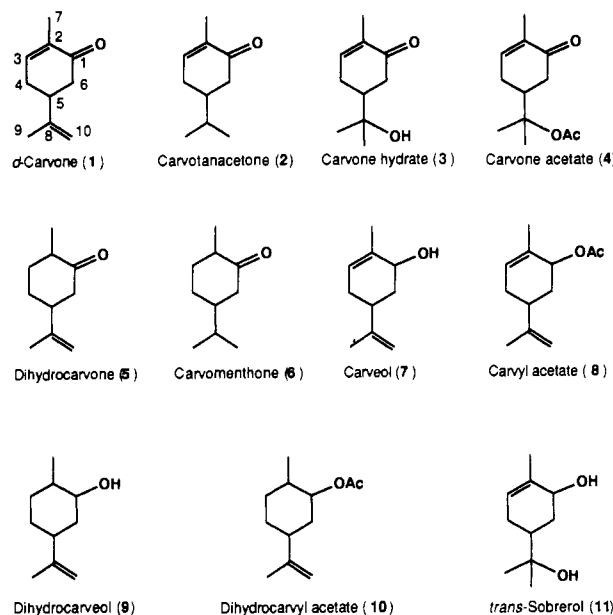

Figure 1. Structures of carvone compounds 1–11.

Carvomenthone (6). Dihydrocarvone (5, 2.0 g, 13.2 mmol) dissolved in methanol (10 mL) was hydrogenated in the presence of 10% palladium on activated carbon (0.5 g) overnight at room temperature and normal pressure. The catalyst was removed by filtration through a Celite pad and washed with methanol. The combined filtrate and washings were evaporated, and the crude product was purified by distillation under reduced pressure to give compound 6 as a colorless oil (1.8 g, 11.7 mmol, 89% yield): high-resolution EI-MS calcd for C$_{10}$H$_{18}$O (M$^+$) 154.1358, obsvd 154.1363; ^{1}H NMR (CDCl$_3$) δ 0.84 (m, 6 H, 9-CH$_3$ and 10-CH$_3$ of the isopropyl group), 0.96 (d, 3 H, J = 6.6 Hz, 7-CH$_3$), 1.15–1.70 (m, 5 H, H-8, 3-CH$_2$ and 4-CH$_2$), 1.80 (m, 1 H, H-5), 1.94–2.44 (m, 3 H, H-2 and 6-CH$_2$); ^{13}C NMR (CDCl$_3$) δ 14.28 (C-7), 19.52 (C-9 and C-10), 28.81 (C-4), 32.66 (C-3), 35.00 (C-8), 44.80 (C-2), 45.28 (C-5), 46.49 (C-6), 213.52 (C-1); EI-MS m/z (relative intensity) 154 (M$^+$, 33), 111 (M$^+$ – C$_3$H$_7$, 100), 95 (M$^+$ – C$_3$H$_7$ – CH$_4$, 20), 83 (M$^+$ – C$_3$H$_7$ – CO, 22), 69 (m/z 95-C$_2$H$_2$, 20), 55 (m/z 83-C$_2$H$_4$, 60), 41 (C$_3$H$_5^+$, 31).

Animals. Female A/J mice, 6 weeks of age, were obtained from the Harlan Sprague Dawley Co. (Indianapolis, IN). Animals were housed in temperature-controlled animal quarters with a 12/12-h light/dark cycle. They were acclimated for 1 week after arrival before they were fed semipurified diet (ICN Nutritional Biochemicals, Cleveland, OH) until the end of the experiment. This diet was similar in composition to that of AIN-76A except the antioxidants were removed and the sucrose was replaced by 1:1 mixture of starch and glucose. Water was given ad libitum. One week after the start of the semipurified diet, they were divided into experimental and control groups with four mice per group. The experimental groups were given by gavage 20 mg per dose of the test compounds, suspended in 0.3 mL of cottonseed oil, once every 2 days for a total of three doses. The control group was given cottonseed oil alone. Twenty-four hours after the last administration, the mice were killed by cervical dislocation and the liver, forestomach, lung, and the mucosa of the proximal one-third of the small bowel and the large bowel including the cecum were removed for enzyme preparation. The tissues were homogenized in cold 1.15% KCl solution (pH 7.4) by means of a Brinkman homogenizer. The homogenates were centrifuged at 9000g for 20 min, and the supernatants were centrifuged at 100000g for 1 h. The cytosolic fractions were kept frozen at –80 °C until use. Each sample represents one tissue from each individual animal. The protein concentration of these samples were determined according to the method of Lowry and co-workers (Lowry et al., 1951).

Glutathione *S*-Transferase Assay. The activity of cytosolic GST was assayed according to the method of Habig and co-workers using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig et al., 1974). Assays were performed at 30 °C in 0.1 M phosphate buffer (pH 6.5), in the presence of 5 mM

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Table I. Effects of Carvone Compounds on the Activity of Glutathione S-Transferase in Target Tissues of A/J Mice

chemical[e]	liver GST sp act.[c]	liver ratio[d]	small bowel mucosa GST sp act.[c]	small bowel mucosa ratio[d]	forestomach GST sp act.[c]	forestomach ratio[d]	lung GST sp act.[c]	lung ratio[d]	large bowel mucosa GST sp act.[c]	large bowel mucosa ratio[d]
control	3.02 ± 0.66		1.41 ± 0.86		1.23 ± 0.47		0.49 ± 0.19		0.27 ± 0.10	
d-carvone (1)	7.08 ± 1.11	2.34[a]	3.24 ± 0.63	2.30[b]	4.53 ± 0.97	3.68[b]	0.68 ± 0.05	1.39	0.71 ± 0.25	2.63[b]
dihydrocarvone (5)	5.73 ± 0.90	1.90[a]	2.04 ± 0.21	1.45	1.61 ± 0.17	1.31	0.50 ± 0.06	1.02	0.52 ± 0.22	1.93
carvomenthone (6)	4.24 ± 0.88	1.41[b]	1.06 ± 0.31	0.75	1.34 ± 0.46	1.09	0.26 ± 0.11	0.96	0.53 ± 0.08	1.08
carveol (7)	5.75 ± 0.42	1.90[a]	2.25 ± 0.25	1.60	2.10 ± 0.31	1.71[b]	0.60 ± 0.20	1.22	0.51 ± 0.23	1.89
carvyl acetate (8)	4.41 ± 0.29	1.46[b]	1.60 ± 0.15	1.13	1.65 ± 0.35	1.34	0.58 ± 0.07	1.18	0.54 ± 0.21	2.00
dihydrocarveol (9)	4.30 ± 0.13	1.42[b]	1.43 ± 0.13	1.01	1.82 ± 0.15	1.48	0.49 ± 0.07	1.00	0.36 ± 0.22	1.33
dihydrocarveol acetate (10)	4.01 ± 0.71	1.33[b]	1.00 ± 0.55	0.71	1.59 ± 0.50	1.09	0.49 ± 0.06	1.00	0.28 ± 0.23	1.04
control	1.38 ± 0.08		0.48 ± 0.04		1.07 ± 0.12		0.36 ± 0.07		0.19 ± 0.05	
d-carvone (1)	4.46 ± 0.41	3.23[a]	3.06 ± 0.48	6.38[a]	2.70 ± 0.74	2.52[a]	0.46 ± 0.06	1.28	0.47 ± 0.17	2.47[b]
carvotanacetone (2)	3.94 ± 0.50	2.86[a]	2.04 ± 0.24	4.25[a]	2.16 ± 0.21	2.02[a]	0.46 ± 0.06	1.14	0.24 ± 0.05	1.26
carvone hydrate (3)	2.01 ± 0.37	1.46[a]	0.93 ± 0.41	1.94	2.08 ± 0.33	1.94[a]	0.48 ± 0.05	1.33[b]	0.22 ± 0.02	1.16
carvone acetate (4)	3.90 ± 0.31	2.83[a]	1.22 ± 0.14	2.54[a]	1.99 ± 0.11	1.86[a]	0.41 ± 0.09	1.14	0.24 ± 0.01	1.26
trans-sobreol (11)	1.41 ± 0.15	1.02	0.47 ± 0.16	0.98	1.06 ± 0.13	0.99	0.39 ± 0.06	1.08	0.22 ± 0.04	1.16

[a] All P values were obtained by Student's t-test ($n = 4$) compared to the control, $P < 0.005$. [b] $P < 0.05$. [c] μmol min^{-1} (mg of protein)$^{-1}$. [d] Test/control. [e] The dosage for all carvone compounds was 20 mg in 0.3 mL of cottonseed oil per mouse given every other day for a total of three doses. The control groups were given 0.3 mL of cottonseed oil only.

Table II. Effects of Carvone Compounds on the Acid-Soluble Sulfhydryl Levels in Target Tissues of A/J Mice

chemical[e]	liver SH concn[c]	liver ratio[d]	small bowel mucosa SH concn[c]	small bowel mucosa ratio[d]	forestomach SH concn[c]	forestomach ratio[d]	lung SH concn[c]	lung ratio[d]	large bowel mucosa SH concn[c]	large bowel mucosa ratio[d]
control	9.49 ± 3.20		10.63 ± 2.28		1.17 ± 0.63		2.54 ± 1.58		3.76 ± 2.80	
d-carvone (1)	3.41 ± 1.26	0.36[a]	9.44 ± 1.75	0.89[b]	2.99 ± 2.29	2.55[b]	1.31 ± 0.55	0.52[b]	2.51 ± 0.36	0.67[b]
dihydrocarvone (5)	14.89 ± 2.32	1.57[b]	10.33 ± 1.68	0.97	3.44 ± 2.34	2.93[b]	2.15 ± 0.35	0.85	4.90 ± 1.83	1.30
carvomenthone (6)	13.55 ± 1.41	1.43[b]	11.06 ± 0.38	1.04	4.00 ± 1.88	3.41[a]	1.74 ± 0.81	0.69	3.39 ± 1.53	0.90
carveol (7)	14.67 ± 1.87	1.55[b]	12.10 ± 1.76	1.14	1.65 ± 0.90	1.40	1.94 ± 0.50	0.76	4.76 ± 1.41	1.27
carvyl acetate (8)	13.23 ± 1.08	1.39	10.11 ± 1.03	0.95	2.62 ± 0.45	2.24	1.73 ± 0.49	0.68	5.11 ± 1.07	1.36
dihydrocarveol (9)	13.59 ± 1.39	1.43	10.71 ± 0.87	1.01	1.42 ● 0.57	1.21	2.10 ± 0.12	0.82	4.29 ± 1.21	1.14
dihydrocarveol acetate (10)	12.80 ± 4.52	1.35	11.29 ± 5.51	1.06	1.64 ± 1.63	1.40	1.53 ± 0.59	0.60[b]	4.29 ± 0.87	1.14
control	13.69 ± 0.84		6.87 ± 0.73		3.43 ± 0.75		2.48 ± 0.26		3.10 ± 1.87	
d-carvone (1)	5.26 ± 3.08	0.38[a]	10.60 ± 1.01	1.54[b]	4.67 ± 2.25	1.36	1.52 ± 0.86	0.61[b]	1.89 ± 1.37	0.61[b]
carvotanacetone (2)	14.02 ± 1.24	1.03	11.06 ± 1.67	1.61[b]	5.22 ± 1.23	1.52	2.62 ± 0.20	1.06	2.99 ± 0.87	0.97
carvone hydrate (3)	11.15 ± 2.80	0.81[b]	9.53 ± 0.71	1.39	7.75 ± 1.28	2.26[b]	2.47 ± 0.42	1.00	2.34 ± 0.67	0.75[b]
carvone acetate (4)	14.53 ± 0.26	1.06	10.78 ± 1.07	1.57[b]	6.49 ± 2.09	1.89	2.36 ± 0.43	0.95	2.16 ± 1.07	0.70[b]
trans-sobreol (11)	10.90 ± 0.68	0.80[b]	7.45 ± 1.03	1.09	7.15 ± 0.94	2.08	1.91 ± 0.69	0.77	3.55 ± 2.48	1.15

[a] All P values were obtained by Student's t-test ($n = 4$) compared to the control, $P < 0.005$. [b] $P < 0.05$. [c] μmol/g of tissue. [d] Test/control. [e] The dosage for all carvone compounds was 20 mg in 0.3 mL of cottonseed oil per mouse given every other day for a total of three doses. The control groups were given 0.3 mL of cottonseed oil only.

GSH, 1 mM CDNB, and 20 μL of the cytosol. The reaction was monitored at 340 nm on a Beckman DU-65 spectrophotometer equipped with a temperature-controlled cell compartment. Complete assay mixture without the cytosolic enzyme was used as the control. Data were analyzed by Student's t-test, and P values were obtained in comparison to the control.

Determination of Acid-Soluble Sulfhydryl Level. The acid-soluble SH level in tissue homogenates was assayed according to the method of Ellman (1959). Aliquots of tissue homogenates were precipitated with equal volumes of 4% sulfosalicylic acid. The supernatants were assayed for the presence of free SH groups by the addition of 9× volume of Ellman's reagent [0.1 mM 5,5'-dithiobis(2-nitrobenzoic acid)] in 0.1 M sodium phosphate buffer (pH 8.0). The absorbance was recorded at 412 nm on the UV spectrophotometer.

RESULTS

A total of 11 carvone-type compounds (Figure 1) were tested for their ability to induce increased activity of GST in the liver, lung, forestomach, and small and large bowel mucosa of female A/J mice (Table I). Carvone (1) was used as a standard for the comparison of the data obtained from two different experiments. Of these compounds, d-carvone appeared to be the most active in the induction of increased GST activity in all five tissues examined. With carvone, the GST activity in the forestomach cytosols was increased to greater than 3.5 times the control level. In the cytosols of liver and small and large bowel mucosa, the GST enzyme activity was significantly higher (>2.0) than that of the control level. However, only a small increase of enzyme activity in the lung cytosols was observed.

Compounds 2-6 that contain the intact ketone group or α,β-unsaturated carbonyl system increased GST activity significantly in the liver. Furthermore, the α,β-unsaturated carbonyl compounds 2-4 appeared to be more active than the simple ketone compounds in all tissues except the large intestinal mucosa. Similar to the parent compounds, no significant change of GST activity in the lung was observed for this group of compounds. Compounds 7-11 whose carbonyl group was reduced appeared to be less active than the corresponding ketone compounds. This group of compounds did not increase GST activity greater than 2 times the control level in any of the tissues examined. In this group, carveol and its acetate exhibited higher activity in most of the tissues than the rest of the compounds.

The effects of carvone compounds on the glutathione level was determined in the five tissues of the female A/J mice (Table II). The forestomach showed maximal responses to these compounds. Carvone hydrate (3), dihydrocarvone (5), carvomenthone (6), carvyl acetate (8), and trans-sobrerol (11) increased the GSH level significantly in the forestomach ($T/C > 2.0$). However, none of the compounds tested elevated GSH level significantly in the other four tissues ($T/C < 1.7$). It is interesting to note that d-carvone caused a decrease in GSH level in the liver ($T/C = 0.36$), lung ($T/C = 0.52$), colon ($T/C = 0.67$), and small intestinal mucosa ($T/C = 0.89$).

DISCUSSION

The present results indicated that several carvone compounds are capable of inducing increased activity of the detoxifying enzyme GST when given orally. Previous studies have shown that compound 1, which was found to inhibit nitrosamine-induced carcinogenesis in mice (Wattenberg et al., 1989), induced increased GST activity significantly higher than that of the control (Lam and Zheng, 1991a). In this SAR study, none of the compounds tested were found to be more potent than d-carvone in any tissues examined. In our previous experiment d-carvone showed higher activity than l-carvone toward the induction of GST activity (unpublished results). In addition, the α,β-unsaturated ketone system appeared to be required for the maintenance of high enzyme-inducing activity of carvones. Saturation of the conjugated olefinic bond (compounds 5 and 6) or reduction of the carbonyl group (compounds 7 and 8) would decrease activity significantly (Table I). If more than one functional group, for instance, both double bond and ketone function from the conjugated system, were changed, the activity was almost lost (compounds 9 and 10). The isolated olefinic double bond of the isopropenyl side chain in the molecule appeared to be important but not so critical as the enone conjugated system for the activity. A decrease in the GST-inducing activity was observed upon reduction of the isopropenyl group (1 vs 2 or 5 vs 6) or conversion of the double bond to an alcohol (1 vs 3 or 7 vs 11). This suggests that the isopropenyl group may have some effects on the interaction between carvone compounds and the enzyme or receptor. It has not been determined whether the induction of GST by this type of compound is a result of stimulation of enzyme protein synthesis or change of the enzyme conformation. Talalay and co-workers, however, have proposed that many anticarcinogenic enzyme inducers are Michael reaction acceptors characterized by olefinic bonds conjugated with electron-withdrawing groups. The potency of inducers parallels their efficiency in Michael reactions (Talalay et al., 1988). Our finding appears to be consistent with such a hypothesis.

The acid-soluble SH level is a measure of the glutathione content in tissues. This important tripeptide thiol is present in most cells. An increase of GSH level is considered an important event in the protection of cells against xenobiotics. The test results indicated that d-carvone caused a decrease in GSH levels in most of the mouse tissues, particularly in the liver, while other compounds appeared to have no significant effect on the target tissues except the forestomach (Table II), where several compounds increased GSH levels to greater than 2 times the control level. In the chemopreventive sense, when normal tissues are being treated, stimulation of the GST activity is a detoxifying mechanism to eliminate carcinogenic substances. Thus, in the case of d-carvone, although it decreased cell GSH levels, the overall effects on the detoxifying system (GST and GSH) favor the elimination of xenobiotics. For most of the other compounds, the GSH-elevating activity is not consistent with the GST-inducing activity or not related to the structure–activity relationship, which suggested a different mechanism for the induction of increased GSH level.

The relationship between intracellular GSH content and the cellular response to antineoplastic drugs has been reviewed by Russo and co-workers (Russo et al., 1986). A number of studies have identified GSH elevations in tumor cells expressing innate or acquired drug resistances (DeVries et al., 1989; Evans et al., 1987; Lewis et al., 1988) and indicated that reduction in intracellular GSH content

of tumor cells can increase drug sensitivity (Ublacker et al., 1991; Green et al., 1984; Mitchell and Russo, 1987). L-Buthionine (SR)-sulfoximine (BSO) is a well-known GSH-decreasing agent, which is being tested for the treatment of drug resistance of tumor cells. BSO can increase the tumor cell sensitivity to chemotherapeutic drugs by depleting GSH in the tumor cells, which reduces the rate of drug elimination. It has been proposed as an adjuvant with other chemotherapeutic agents. It was reported that BSO decreased GSH levels significantly in several mouse tissues including the liver, where a 52% decrease was observed (Campbell et al., 1991) compared to the 64% decrease by d-carvone. Since the GSH depletion activity of d-carvone was found to be comparable to that of BSO, it may be useful as a lead compound for the development of adjuvant agents of cancer chemotherapy.

Most of the essential oils are used commercially as flavoring agents in food, cosmetics, detergents, and other products, which are being consumed by or come in contact with the general population. d-Carvone is the major constituent in a number of common essential oils (Formacek and Kubeczka, 1982), such as caraway oil (43%) and dillweed oil (27%), and is responsible for the GST-inducing activity observed with these oils. Since induction of GST activity is correlated with anticarcinogenic activity, this type of compound can be considered a potential chemopreventive agent.

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