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EFFECTS OF CYCLIC 12-, 8-, AND 6-CARBON COMPOUNDS ON GLUTATHIONE S-TRANSFERASE ACTIVITY

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The effects of feeding ICR/Ha mice cyclic 12-, 8-, and 6-carbon compounds on glutathione S-transferase (GST) activity in the liver, intestinal mucosa, and the forestomach were determined. The compounds used for this study were 1,5,9-trans,trans,cis-cyclododecatriene, 1,2-trans-5,6-trans-9,10-cis-cyclododecatriene-1,2-oxide, cyclododecanol, cyclododecene oxide, cyclododecane, 1,5-cyclooctadiene, cyclooctene oxide, cyclohexene, and cyclohexene oxide. The unsaturated cyclic 12-carbon compounds elicited the greatest increase in GST activity. Thus, feeding 1,5,9-trans,trans,cis-cyclododecatriene increased this activity almost 4-fold in the livers and the intestinal mucosa of experimental animals. Cyclic 8-carbon compounds were less effective and feeding the cyclic 6-carbon compounds did not result in any significant increase in GST activity. None of the compounds elicited increased GST activity in the forestomach. Previous studies have shown that compounds inducing increased GST activity can protect against chemical carcinogens. It remains to be determined whether the compounds identified in the present investigation as inducers of this enzyme system will have such protective capacities.

Glutathione S-transferase (EC 2.5.1.18), has been studied extensively as a major detoxification system which catalyzes conjugation of a wide variety of electrophilic compounds to glutathione (1,2). Since most of the reactive forms of chemical carcinogens have strong electrophilic sites, GST activity has a very important role in the detoxification of carcinogens. Compounds which enhance this activity have been shown to provide protection against chemical carcinogenesis. For example, compounds that increase the GST activity of the mouse forestomach by approximately two-fold or more have been found to inhibit the occurrence of benzo(a)pyrene-induced neoplasia of that structure (3). Compounds found to enhance GST activity and to inhibit carcinogenesis have widely varied structures. They include phenols, lactones, coumarins, aromatic isothiocyanates, nonpolar flavones and dithiolthiones (4,5,6). Natural products that induce increased GST activity also have been found to

Abbreviation: GST, glutathione S-transferase

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inhibit carcinogenesis. Thus green coffee beans induce increased GST activity and inhibit mammary tumor formation in rats resulting from administration of 7,12-dimethylbenz(a)anthracene. Two potent inducers of increased GST activity have been identified from these beans. They are the diterpene esters, kahweol palmitate and cafestol palmitate (8).

In the present study, the effects of feeding ICR/Ha mice monocyclic hydrocarbons with different chain lengths, varied saturation and also feeding some of their oxygenated derivatives were probed to ascertain if compounds of this type would elicit increased GST activity.

MATERIALS AND METHODS

Animal Experiments. Female ICR/Ha mice (Madison, WI colony of Harlan-Sprague-Dawley, Indianapolis, IN) were used in all experiments. At 7 weeks of age mice were randomized by weight and divided into groups of 5-10 mice. The control group received a semipurified diet consisting of 27% vitamin-free casein, 59% starch, 10% corn oil, 4% salt mix (U.P.S. XIV), and a complete mixture of vitamins (Teklad, Inc., Madison, WI). Other groups received this semipurified diet supplemented with the test compounds at concentrations of either 30 or 50 μmoles/g diet. The diets were fed for 2 weeks. The mice were then killed and the livers, forestomach, and the mucosa from the small bowel removed, homogenized in 0.1 M phosphate buffer, pH 7.5, and centrifuged at 100,000 x g for an hour. The supernatant was used for determining GST activity using 1-chloro-2,4-dinitrobenzene as substrate (9).
Chemicals. Cyclohexene, cyclohexene oxide, cyclododecanol, cyclododecene oxide (cyclododecane epoxide), 1,5,9-trans,trans,cis-cyclododecatriene, 1,5-cyclooctadiene, cyclooctene oxide, and 1-chloro-2,4-dinitrobenzene were obtained from Aldrich Chemical Company; 1,2-trans,5,6-trans,9,10-cis-cyclododecatriene-1,2-oxide (1,2-epoxy-5,6-trans-9,10-cis-cyclododecadiene) from Aldrich Alfa-Bader; and cyclododecane from Dupont.

RESULTS AND DISCUSSION

GST activity in the livers and in the mucosa of small intestine were determined in tissues from animals that received the test compounds or control diet as described above. The chemical structures of the test compounds studied are shown in Fig. 1. The ratios of the GST activity of tissues from animals on experimental diets as compared to this activity in the control animals are shown in Table I. The activity of GST in the livers and the intestinal mucosa were considerably increased in the animals fed the unsaturated 12-carbon cyclic compounds. Increases in GST activity of 3.79-, 3.78- and 3.82-fold were observed in the three experiments, respectively, in the livers of animals fed the 1,5,9-trans,trans,cis-cyclododecatriene (I). The increases in the intes-

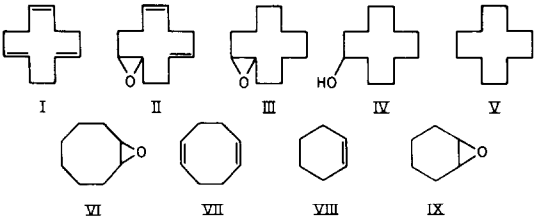

Figure 1. Chemicals used in the study. 1,5,9-trans,trans,cis-cyclododecatriene (I), 1,2-trans-5,6-trans-9,10-cis-cyclododecatriene-1,2-oxide (II), cyclododecene oxide (III), cyclododecanol (IV), cyclododecane (V), cyclooctene oxide (VI), 1,5-cyclooctadiene (VII), cyclohexene (VIII), and cyclohexene oxide (IX).

tinal GST activity were of a similar magnitude. The corresponding unsaturated oxide (II) produced increases of 2.91- and 3.81-fold in the livers and the intestinal mucosa, respectively, but the corresponding saturated oxide (III)

Table 1. Effects of feeding test chemicals on the glutathione S-transferase activity in the cytosols of liver and small bowel mucosa

Experiment	Test Compound		Liver		Small Bowel Mucosa	
			Specific Activity	Ratio	Specific Activity	Ratio
1	None		1.96 ± 0.16		0.61 ± 0.04	
	1,5,9-trans,trans, cis-cyclododecatriene	(I)	7.42 ± 0.53*	3.79 ± 0.42	2.77 ± 0.29*	4.54 ± 0.58
	cyclododecene oxide (III)		4.90 ± 0.52*	2.50 ± 0.34	1.23 ± 0.04*	2.02 ± 0.16
	cyclododecanol IV)		3.21 ± 0.11*	1.64 ± 0.15	1.07 ± 0.02*	1.75 ± 0.13
	cyclooctene oxide (VI)		3.32 ± 0.32*	1.69 ± 0.22	0.68 ± 0.01**	1.11 ± 0.08
	1,5-cyclooctadiene (VII)		2.75 ± 0.26*	1.40 ± 0.18	0.76 ± 0.03*	1.25 ± 0.10
2	None		2.40 ± 0.10		0.65 ± 0.01	
	1,5,9-trans,trans, cis-cyclododecatriene	(I)	9.06 ± 0.04*	3.78 ± 0.15	1.74 ± 0.15*	2.68 ± 0.23
	cyclohexene (VIII)		2.64 ± 0.16**	1.10 ± 0.08	0.70 ± 0.06	1.08 ± 0.10
	cyclohexene oxide (IX)		2.34 ± 0.10	0.98 ± 0.06	0.67 ± 0.03	1.03 ± 0.04
3	None		1.39 ± 0.05		0.27 ± 0.01	
	1,5,9-trans,trans, cis-cyclododecatriene	(I)	5.31 ± 0.23*	3.82 ± 0.21	1.10 ± 0.09*	4.07 ± 0.36
	1,2-trans-5,6-trans-9, decatriene-1,2-oxide	(II)	4.05 ± 0.38*	2.91 ± 0.29	1.03 ± 0.11*	3.81 ± 0.44
	10-cis-cyclodo cyclododecane	(V)	1.84 ± 0.13*	1.32 ± 0.10	0.53 ± 0.04*	1.96 ± 0.15

Glutathione S-transferase activity was determined spectrophotometrically at 30° with 1-chloro-2,4-dinitrobenzene as substrate (9). Specific activities are expressed as μmoles per min. per mg protein. They represent the mean value obtained in three determinations. Standard deviations are also shown.

* $p < 0.005$
** $p < 0.05$

was somewhat less effective. It produced 2.50- and 2.02-fold increases in GST activity in the liver and the intestinal mucosa, respectively. Cyclododecanol (IV) and cyclododecane (V) were less effective than the corresponding oxide (III). The 8-carbon compounds, cyclooctene oxide (VI) and 1,5-cyclooctadiene (VII), also produced some increases in GST activity in these tissues, but feeding the 6-carbon compounds did not result in any significant increases.

GST activity was also determined in the forestomachs of control and experimental animals. There were no significant differences in the activity of this enzyme in the forestomach of experimental animals as compared to the controls. This activity in the control animals was 1.22 ± 0.04 μmol/min/mg protein.

Thus, our experiments showed that the unsaturated cyclic 12-carbon compounds were the most effective in producing increased GST activity in the liver and the intestinal mucosa. The saturated compounds were less effective. The oxide was more effective than the corresponding alcohol. Saturated hydrocarbon was the least effective. The 8-carbon compounds were less effective than the corresponding 12-carbon compounds. The 6-carbon compounds were ineffective.

Since the unsaturated 12-carbon compounds were quite efficient in eliciting increased GST activity it will be of great interest to find out whether they can provide any protection against chemical carcinogenesis.

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