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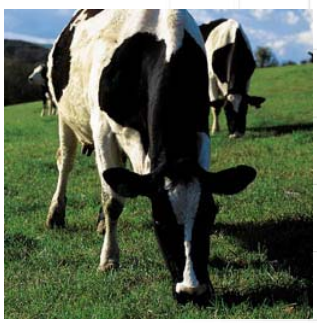
# Structural requirements for induction of cancer-protective enzymes by isothiocyanates

Report to Waikato Medical Research Foundation

FBP 12553

May 2012



Client Report – FBP 12553

# Structural requirements for induction of cancer-protective enzymes by isothiocyanates

## Waikato Medical Research Foundation

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May 2012

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## Summary

Humans are continually exposed to carcinogens, which are present in the atmosphere and in our food, and which are produced in our bodies by the natural processes of metabolism. But we do not all develop cancer, because we are protected against carcinogens by a family of enzymes that convert them to unreactive metabolites, which are readily excreted from the body. These are the Phase II detoxification enzymes, and there is evidence from animal experiments that, after exposure to carcinogens, cancer occurs only when these enzymatic defences are overwhelmed. If we could increase the strength of our defences against carcinogens by increasing the levels of these beneficial enzymes in our tissues, we could decrease the likelihood of developing cancer.

Bladder cancer is a major human health problem. In Western countries, it is the fourth commonest cancer in men and the eighth commonest in women. Epidemiological studies have shown that the incidence of bladder cancer is lower in individuals who consume large amounts of Brassica vegetables, such as cabbage, cauliflower, broccoli and Brussels sprouts. The protection against bladder cancer given by Brassica vegetables is attributable to their ability to form compounds called isothiocyanates when cut or chewed. These compounds are very effective inducers of Phase 2 enzymes in the urinary bladder, and we have shown that sulforaphane, an isothiocyanate derived from broccoli, gives excellent protection against chemically-induced bladder cancer in rats.

Isothiocyanates may thus be useful in protecting against bladder cancer in humans. These compounds vary greatly in their inductive activity, however, and we need to know which would be most effective. More than 120 isothiocyanates occur in nature, and it would be very laborious to test all of these. An understanding of structure-activity relationships would be valuable for selecting isothiocyanates most likely to be effective for cancer chemoprevention.

We have previously shown that a methyl group adjacent to the isothiocyanate moiety increases inductive activity, and that the way in which substituents are arranged around the isothiocyanate group may also have a significant effect. In the present study, we have compared the activities of a number of methylated and isomeric isothiocyanates in inducing Phase 2 enzymes in rat tissues, and have confirmed the effects of methylation and stereochemistry on inductive activity in certain tissues. This work will help in understanding how isothiocyanates interact with the signalling systems that trigger Phase 2 enzyme induction, and may lead to the development of substances with exceptionally high activity, which would be of great value in cancer chemoprevention.

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# 1. Introduction

There is evidence that the incidence of cancer in humans could be decreased by changes in lifestyle, with dietary modifications being particularly important (1). The recent report (2) by the World Cancer Research Fund/American Institute for Cancer Research "Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective" emphasises the fact that individuals consuming diets high in fruit and vegetables are less at risk of developing cancer than those with a relatively low intake of these foodstuffs.

Most human cancers result from the irreversible interaction of cancer-causing chemicals (carcinogens) with DNA. We are continually exposed to carcinogens in our food and in the atmosphere, while others are produced within our bodies as normal products of cellular metabolism. We do not all develop cancer, however, since we have efficient mechanisms for detoxifying carcinogens, a process that involves the so-called Phase II detoxification enzymes. These enzymes, which include NAD(P)H-quinone reductase (NQO1), glutathione *S*-transferase (GST), epoxide hydrolase and glucuronosyl transferase, convert carcinogens to non-toxic water-soluble materials that are readily eliminated from the body (3, 4). If carcinogen detoxification is rapid, irreversible damage to DNA is avoided, and cancer development prevented.

The efficacy of carcinogen detoxification is proportional to the activities of the Phase II enzymes in tissues. In animal models, an inverse relationship between tissue levels of Phase II enzymes and susceptibility to cancer has been observed (5). Furthermore, genetic changes in humans, leading to deficiencies in Phase II enzyme activity, are associated with increased risk of bladder cancer (6), lung cancer (7) colorectal cancer (8) and leukaemia (9).

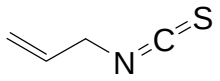
If Phase II enzyme activity in humans could be increased by practicable dietary modifications, cancer incidence may be decreased. In work previously funded by the Foundation, we have shown that isothiocyanates, which are compounds derived from Brassica vegetables, increase tissue activities of Phase II enzymes in rats. The greatest effect of isothiocyanates was observed in the urinary bladder (10-12), suggesting that these substances, and the vegetables from which they are derived, would be especially effective in protecting against bladder cancer. This is in accord with epidemiological evidence (13) and we have shown that an isothiocyanate from broccoli gave excellent protection against chemically-induced bladder cancer in rats (14).

Bladder cancer is an important problem. In Western countries, it is the fourth commonest cancer in men and the eighth commonest in women (15). Treatment for bladder cancer continues to improve, but recurrence occurs in 60-75% of patients (16), so that repeated diagnostic evaluation and therapy are required, resulting in

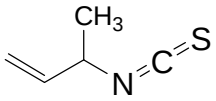
considerable patient discomfort and economic burden. The median age of first diagnosis of bladder cancer is 69 for men and 71 for women (17). The life expectancy of men in New Zealand is 78 years, and that of women 82 years (18) so that if the onset of disease could be delayed by 10 years by use of chemopreventative agents, the incidence of bladder cancer would be greatly diminished. Isothiocyanates from vegetables could play a major role in this process.

More than 120 different isothiocyanates have been shown to be present in Brassica vegetables (19), but only a small number have been tested for their ability to increase tissue activities of Phase 2 enzymes. Previous studies have shown that there are very large variations in the inductive activity of different isothiocyanates, and that certain aspects of the chemical structure of these compounds have a major influence on their inductive activity.

We have previously shown (20) that a methyl group on the carbon atom adjacent to the isothiocyanate group increases inductive activity. For example, allyl isothiocyanate [I] at a dose of 250 μmoles/kg/day for 5 days caused a 5.5-fold increase in NQO1 in the urinary bladder, while the methylated compound 1-methylallyl isothiocyanate (II) increased the activity of this enzyme by a factor of 7.8.

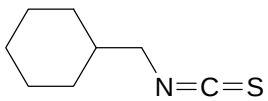


[I]

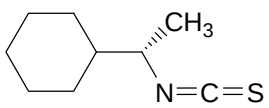


[II]

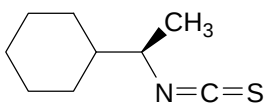
We have also shown that cyclohexylmethyl isothiocyanate (III) is a very good inducer of Phase 2 enzymes in the urinary bladder, and it would be expected that the compound substituted with a methyl group on the carbon next to the isothiocyanate group would be even better. This compound is 1-cyclohexylethyl isothiocyanate, which exists in 2 isomeric forms, the R-(-)-enantiomer (IV), in which the methyl group lies below the plane of the ring and the S-(+) enantiomer (V), in which it lies above the ring.



[III]



[IV]



[V]

The results of a preliminary experiment with these two enantiomers were rather surprising. The R-(-)-isomer was a better inducer than cyclohexylmethyl isothiocyanate, as expected. But the S-(+) isomer was a much weaker inducer, and less effective than the un-methylated compound.

In the present study, we have examined several more substituted isothiocyanates for their effects on Phase 2 enzyme activity in various tissues of the rat.

## 2. Methods

Six female Sprague-Dawley rats, 10-12 weeks old at the start of the experiment, were dosed by gavage with the isothiocyanates listed below at 250 μmoles/kg/day for 5 days. On the sixth day, the animals were euthanased and tissues harvested for determination of levels of NQO1 and GST.

Compounds tested:

*Experiment 1*. The two enantiomers of 1-phenylethyl isothiocyanate, compared with benzyl isothiocyanate, which lacks the methyl group next to the isothiocyanate moiety.

*Experiment 2.* The two enantiomers of 1-phenylpropyl isothiocyanate, compared with 2-phenylethyl isothiocyanate, which lacks the methyl group next to the isothiocyanate moiety.

*Experiment 3.* The two enantiomers of 2-hexyl isothiocyanate, compared with 1-pentyl isothiocyanate, which lacks the methyl group next to the isothiocyanate moiety.

*Experiment 4.* The two enantiomers of 2-heptyl isothiocyanate, compared with 1-hexyl isothiocyanate, which lacks the methyl group next to the isothiocyanate moiety.

# 3. Results

The relative activities of NQO1 in the various rat tissues are shown in Tables 1 and 2; those of GST are given in Tables 3 and 4.

The results of the assays of bladder Phase 2 enzyme activities were not consistent with those previously obtained with the enantiomers of 1-cyclohexylethyl isothiocyanate, with which the inductive activity of the R-(-) enantiomer was significantly greater than that of the S-(+) enantiomer. With 1-phenylethyl isothiocyanate, 1-phenylpropyl isothiocyanate, 2-heptyl isothiocyanate and 2-heptyl isothiocyanate, increases in NQO1 and GST in the urinary bladder were no higher with the R-(-) enantiomer than the S-(+) enantiomer, and in the case of the first-named compound, the effect was significantly lower. Furthermore, the compounds with a methyl group adjacent to the isothiocyanate group were no more effective in the urinary bladder than those lacking this moiety.

In contrast, in the forestomach, glandular stomach and small intestine the inductive activities of the R-(-) enantiomers were in most cases greater than those of the S-(+) enantiomers and also greater than those of the un-methylated isothiocyanates. This effect was particularly pronounced in the jejunum and ileum.

None of the isothiocyanates tested in these experiments had a pronounced effect on Phase 2 enzyme activity in the liver, caecum, colon, lung or spleen.

**Table 1.** Effect of isothiocyanates on the relative activities of NQO1 in rat tissues. Experiments 1 and 2.

| Exp No | Compound | Urinary bladder | Fore-stomach | Glandular stomach | Duodenum | Jejunum | Ileum | Caecum | Colon | Liver | Kidney | Lung | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (R)-(-)-1-Phenylethyl | 2.27 ± 0.28$^{a}$ | 1.44 ± 0.15$^{a}$ | 1.86 ± 0.19$^{a}$ | 6.12 ± 0.35$^{a}$ | 8.03 ± 0.41$^{a}$ | 4.87 ± 0.30$^{a}$ | 2.09 ± 0.07$^{a}$ | 2.20 ± 0.11$^{a}$ | 2.11 ± 0.11$^{a}$ | 2.08 ± 0.20$^{a}$ | 1.55 ± 0.07$^{a,b}$ | 2.61 ± 0.09$^{a}$ |
| 1 | (S)-(+)-1-Phenylethyl | 4.93 ± 0.63$^{b}$ | 1.22 ± 0.14$^{a}$ | 0.84 ± 0.09$^{b}$ | 3.12 ± 0.18$^{b}$ | 3.26 ± 0.13$^{b}$ | 2.85 ± 0.20$^{a}$ | 1.41 ± 0.08$^{b}$ | 1.83 ± 0.10$^{a}$ | 1.72 ± 0.14$^{b}$ | 1.33 ± 0.12$^{b}$ | 1.30 ± 0.11$^{b}$ | 1.52 ± 0.15$^{a,b}$ |
| 1 | Benzyl | 7.96 ± 0.18$^{c}$ | 1.57 ± 0.12$^{a}$ | 1.78 ± 0.26$^{a}$ | 5.22 ± 0.05$^{a}$ | 5.14 ± 0.13$^{c}$ | 3.41 ± 0.32a | 1.54 ± 0.08$^{b}$ | 1.91 ± 0.17$^{a}$ | 1.40 ± 0.06$^{c}$ | 1.52 ± 0.06$^{a,b}$ | 1.61 ± 0.03$^{a,b}$ | 2.11 ± 0.18$^{a}$ |
| 2 | (R)-(-)-1-Phenylpropyl | 3.85 ± 0.66$^{a}$ | 5.98 ± 0.21$^{a}$ | 1.58 ± 0.27$^{a}$ | 9.73 ± 0.34$^{a}$ | 9.40 ± 0.43$^{a}$ | 5.41 ± 0.17$^{a}$ | 2.16 ± 0.08$^{a}$ | 2.64 ± 0.07$^{a}$ | 2.46 ± 0.08$^{a}$ | 1.79 ± 0.14$^{a}$ | 1.92 ± 0.10$^{a}$ | 2.59 ± 0.22$^{a}$ |
| 2 | (S)-(+)-1-Phenylpropyl | 3.10 ± 0.32$^{a}$ | 2.10 ± 0.23$^{b}$ | 1.19 ± 0.15$^{a}$ | 3.11 ± 0.44$^{b}$ | 2.76 ± 0.18$^{b}$ | 2.37 ± 0.25$^{b}$ | 1.58 ± 0.08$^{b}$ | 2.27 ± 0.14$^{a}$ | 1.97 ± 0.17$^{b}$ | 1.54 ± 0.15$^{a}$ | 1.41 ± 0.13$^{b}$ | 1.20 ± 0.27$^{b}$ |
| 2 | 2-Phenylethyl | 7.09 ± 0.54$^{b}$ | 4.07 ± 0.28$^{c}$ | 1.48 ± 0.16$^{a}$ | 5.04 ± 0.22$^{c}$ | 3.69 ± 0.34$^{b}$ | 3.20 ± 0.34$^{b}$ | 1.20 ± 0.07$^{c}$ | 1.49 ± 0.11$^{b}$ | 1.31 ± 0.09$^{c}$ | 1.35 ± 0.17$^{a}$ | 1.56 ± 0.14$^{a,b}$ | 1.33 ± 0.08$^{a,b}$ |

Figures shown are the means and SEM of the ratios of the specific activities of treated rats to those of controls. Figures within a column for each of the experiments without the same superscript letter are significantly different at $P<0.05$.

**Table 2.** Effect of isothiocyanates on the relative activities of NQO1 in rat tissues. Experiments 3 and 4**.**

| Exp No | Compound | Urinary bladder | Fore-stomach | Glandular stomach | Duodenum | Jejunum | Ileum | Caecum | Colon | Liver | Kidney | Lung | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | (R)-(-)-2-Heptyl | 7.41 ± 0.49$^{a}$ | 3.31 ± 0.13$^{a}$ | 1.68 ± 0.11$^{a}$ | 5.61 ± 0.25$^{a}$ | 6.16 ± 0.32$^{a}$ | 4.07 ± 0.27$^{a}$ | 1.97 ± 0.11$^{a}$ | 1.98 ± 0.16$^{a}$ | 1.55 ± 0.21$^{a}$ | 2.59 ± 0.23$^{a}$ | 1.48 ± 0.09$^{a}$ | 2.04 ± 0.09$^{a}$ |
| 3 | (S)-(+)-2-Heptyl | 6.51 ± 0.32$^{a}$ | 2.10 ± 0.06$^{b}$ | 1.12 ± 0.16$^{a}$ | 2.77 ± 0.20$^{b}$ | 2.87 ± 0.61$^{b}$ | 2.57 ± 0.20$^{b}$ | 1.50 ± 0.06$^{b}$ | 1.69 ± 0.08$^{a}$ | 1.40 ± 0.10$^{a}$ | 2.25 ± 0.25$^{a,b}$ | 1.06 ± 0.07$^{b}$ | 1.16 ± 0.17$^{b}$ |
| 3 | 1-Hexyl | 6.78 ± 0.51$^{a}$ | 3.24 ± 0.22$^{a}$ | 1.52 ± 0.17$^{a}$ | 4.06 ± 0.23$^{c}$ | 2.97 ± 0.09$^{b}$ | 2.26 ± 0.13$^{b}$ | 1.20 ± 0.06$^{c}$ | 1.83 ± 0.13$^{a}$ | 1.28 ± 0.08$^{a}$ | 1.54 ± 0.17$^{b}$ | 1.10 ± 0.04$^{b}$ | 1.29 ± 0.13$^{b}$ |
| 4 | (R)-(-)-2-Hexyl | 6.73 ± 0.96$^{a}$ | 4.80 ± 0.42$^{a}$ | 2.29 ± 0.30$^{a}$ | 7.26 ± 0.57$^{a}$ | 7.33 ± 0.40$^{a}$ | 4.61 ± 0.35$^{a}$ | 1.92 ± 0.15$^{a}$ | 2.49 ± 0.20$^{a}$ | 2.08 ± 0.14$^{a}$ | 3.44 ± 0.50$^{a}$ | 1.98 ± 0.18$^{a}$ | 2.22 ± 0.13$^{a}$ |
| 4 | (S)-(+)-2-Hexyl | 7.33 ± 0.55$^{a}$ | 2.72 ± 0.14$^{b}$ | 1.11 ± 0.17$^{b}$ | 2.27 ± 0.07$^{b}$ | 2.24 ± 0.11$^{b}$ | 1.97 ± 0.13$^{b}$ | 1.28 ± 0.05$^{b}$ | 1.43 ± 0.06$^{b}$ | 1.29 ± 0.09$^{b}$ | 1.58 ± 0.12$^{b}$ | 1.26 ± 0.03$^{b}$ | 1.54 ± 0.05$^{b}$ |
| 4 | 1-Pentyl | 6.91 ± 0.27$^{a}$ | 3.54 ± 0.24$^{b}$ | 1.42 ± 0.12$^{b}$ | 3.72 ± 0.24$^{c}$ | 2.90 ± 0.14$^{c}$ | 1.61 ± 0.11$^{b}$ | 1.28 ± 0.05$^{b}$ | 1.18 ± 0.11$^{c}$ | 1.09 ± 0.06$^{b}$ | 1.61 ± 0.40$^{b}$ | 1.15 ± 0.04$^{b}$ | 1.38 ± 0.07$^{b}$ |

Figures shown are the means and SEM of the ratios of the specific activities of treated rats to those of controls. Figures within a column for each of the experiments without the same superscript letter are significantly different at $P<0.05$.

**Table 3.** Effect of isothiocyanates on the relative activities of GST in rat tissues. Experiments 1 and 2.

| Exp No. | Compound | Urinary bladder | Fore-stomach | Glandular stomach | Duodenum | Jejunum | Ileum | Caecum | Colon | Liver | Kidney | Lung | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | (R)-(-)-1-Phenylethyl | 1.22 ± 0.11$^{a}$ | 0.91 ± 0.07$^{a}$ | 1.39 ± 0.12$^{a}$ | 2.35 0.06$^{a}$ | 3.07 ± 0.23$^{a}$ | 2.18 ± 0.20$^{a}$ | 1.36 ± 0.05$^{a}$ | 1.21 ± 0.06$^{a}$ | 1.33 ± 0.08$^{a}$ | 1.12 ± 0.05$^{a}$ | 1.13 ± 0.04$^{a}$ | 1.09 ± 0.03$^{a}$ |
| 1 | (S)-(+)-1-Phenylethyl | 2.31 ± 0.23$^{b}$ | 0.68 ± 0.05$^{b}$ | 0.93 ± 0.02$^{b}$ | 1.90 ± 0.11$^{a}$ | 1.76 ± 0.07$^{b}$ | 1.40 ± 0.07$^{b}$ | 1.21 ± 0.13$^{a,b}$ | 1.05 ± 0.04$^{a}$ | 1.22 ± 0.05$^{a,b}$ | 1.09 ± 0.09$^{a}$ | 1.08 ± 0.09$^{a}$ | 0.97 ± 0.04$^{a}$ |
| 1 | Benzyl | 3.64 ± 0.28$^{c}$ | 0.91 ± 0.05$^{a}$ | 1.12 ± 0.04$^{a,b}$ | 2.04 ± 0.10$^{a}$ | 2.19 ± 0.11$^{b}$ | 1.58 ± 0.13$^{b}$ | 1.13 ± 0.03$^{b}$ | 1.13 ± 0.04$^{a}$ | 1.11 ± 0.03$^{b}$ | 1.07 ± 0.04$^{a}$ | 1.14 ± 0.10$^{a}$ | 1.03 ± 0.03$^{a}$ |
| 2 | (R)-(-)-1-Phenylpropyl | 2.25 ± 0.31$^{b}$ | 2.27 ± 0.13$^{a}$ | 1.37 ± 0.11$^{a}$ | 3.55 ± 0.36$^{a}$ | 3.82 ± 0.11$^{a}$ | 2.30 ± 0.11$^{a}$ | 1.34 ± 0.04$^{a}$ | 1.25 ± 0.02$^{a}$ | 1.72 ± 0.06$^{a}$ | 1.31 ± 0.08$^{a}$ | 1.13 ± 0.04$^{a}$ | 1.17 ± 0.02$^{a}$ |
| 2 | (S)-(+)-1-Phenylpropyl | 1.75 ± 0.12$^{b}$ | 0.87 ± 0.06$^{b}$ | 1.40 ± 0.13$^{a}$ | 2.23 ± 0.19$^{b}$ | 1.99 ± 0.12$^{b}$ | 1.31 ± 0.10$^{b}$ | 1.10 ± 0.04$^{b}$ | 1.22 ± 0.05$^{a}$ | 1.46 ± 0.06$^{b}$ | 1.31 ± 0.10$^{a}$ | 1.13 ± 0.06$^{a}$ | 0.96 ± 0.05$^{a}$ |
| 2 | 2-Phenylethyl | 3.40 ± 0.17$^{c}$ | 1.82 ± 0.11$^{c}$ | 1.14 ± 0.03$^{a}$ | 1.65 ± 0.14$^{b}$ | 1.80 ± 0.21$^{b}$ | 1.64 ± 0.14$^{b}$ | 1.04 ± 0.03$^{b}$ | 1.05 ± 0.08$^{a}$ | 1.27 ± 0.08$^{b}$ | 1.18 ± 0.06$^{a}$ | 1.19 ± 0.05$^{a}$ | 0.98 ± 0.09$^{a}$ |

Figures shown are the means and SEM of the ratios of the specific activities of treated rats to those of controls. Figures within a column for each of the experiments without the same superscript letter are significantly different at $P<0.05$.

**Table 4.** Effect of isothiocyanates on the relative activities of GST in rat tissues. Experiments 3 and 4.

| Exp No. | Compound | Urinary bladder | Fore-stomach | Glandular stomach | Duodenum | Jejunum | Ileum | Caecum | Colon | Liver | Kidney | Lung | Spleen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | (R)-(-)-2-Heptyl | 4.70 ± 0.25$^{a}$ | 1.60 ± 0.08$^{a}$ | 1.47 ± 0.16$^{a}$ | 2.22 ± 0.09$^{a}$ | 2.27 ± 0.14$^{a}$ | 1.80 ± 0.15$^{a}$ | 1.39 ± 0.04$^{a}$ | 1.13 ± 0.08$^{a}$ | 1.37 ± 0.11$^{a}$ | 1.23 ± 0.07$^{a}$ | 1.04 ± 0.05$^{a}$ | 1.20 0.06$^{a}$ |
| 3 | (S)-(+)-2-Heptyl | 4.11 ± 0.38$^{a}$ | 1.05 ± 0.07$^{b}$ | 1.13 ± 0.06$^{a}$ | 1.94 ± 0.18$^{a,b}$ | 1.58 ± 0.11$^{b}$ | 1.36 ± 0.10$^{b}$ | 1.13 ± 0.04$^{b}$ | 1.19 ± 0.17$^{a}$ | 1.25 ± 0.05$^{a}$ | 1.24 ± 0.06$^{a}$ | 1.01 ± 0.06$^{a}$ | 1.01 ± 0.06$^{a}$ |
| 3 | 1-Hexyl | 3.39 ± 0.31$^{b}$ | 1.31 ± 0.07$^{c}$ | 1.13 ± 0.10$^{a}$ | 1.66 ± 0.11$^{b}$ | 1.23 ± 0.08$^{b}$ | 1.18 ± 0.04$^{b}$ | 1.06 ± 0.04$^{b}$ | 1.09 ± 0.06$^{a}$ | 1.09 ± 0.06$^{c}$ | 1.10 ± 0.03$^{a}$ | 1.08 ± 0.03$^{a}$ | 1.07 ± 0.03$^{a}$ |
| 4 | (R)-(-)-2-Hexyl | 5.81 ± 0.55$^{a}$ | 2.23 ± 0.10$^{a}$ | 1.79 ± 0.11$^{a}$ | 2.86 ± 0.32$^{a}$ | 2.67 ± 0.24$^{a}$ | 2.42 ± 0.35$^{a}$ | 1.50 ± 0.11$^{a}$ | 1.21 ± 0.04$^{a}$ | 1.55 ± 0.06$^{a}$ | 1.25 ± 0.03$^{a}$ | 1.17 ± 0.09$^{a}$ | 1.26 ± 0.04$^{a}$ |
| 4 | (S)-(+)-2-Hexyl | 5.23 ± 0.39$^{a}$ | 1.22 ± 0.05$^{b}$ | 1.16 ± 0.06$^{b}$ | 1.40 ± 0.11$^{b}$ | 1.46 ± 0.13$^{b}$ | 1.27 ± 0.05$^{b}$ | 1.18 ± 0.06$^{b}$ | 1.11 ± 0.05$^{a}$ | 1.29 ± 0.03$^{b}$ | 1.29 ± 0.05$^{a}$ | 1.11 ± 0.05$^{a}$ | 1.16 ± 0.04$^{a}$ |
| 4 | 1-Pentyl | 4.18 ± 0.22$^{a}$ | 1.40 ± 0.09$^{b}$ | 1.13 ± 0.06$^{b}$ | 1.42 ± 0.16$^{b}$ | 1.32 ± 0.11$^{b}$ | 1.23 ± 0.09$^{b}$ | 1.11 ± 0.05$^{b}$ | 1.01 ± 0.05$^{a}$ | 1.06 ± 0.05$^{c}$ | 1.16 ± 0.05$^{a}$ | 1.16 ± 0.02$^{a}$ | 1.16 ± 0.05$^{a}$ |

Figures shown are the means and SEM of the ratios of the specific activities of treated rats to those of controls. Figures within a column for each of the experiments without the same superscript letter are significantly different at $P<0.05$.

# 4. Discussion

In view of the earlier results with 1-cyclohexylethyl isothiocyanate, it was expected that all the R-(-) enantiomers would be potent inducers of the Phase 2 enzymes in the urinary bladder. This was not the case. It is possible that the ring system of 1-cyclohexyl isothiocyanate is important for activity, so that the effect would not be seen with the straight-chain compounds of the present study. This is considered unlikely, however, since the R-(-) enantiomers were generally more effective than the S-(+) enantiomers in other tissues, so the lack of effect seems to be restricted to the urinary bladder. It was noted during harvesting of the tissues that the urinary bladders of some rats dosed with the R-(-) enantiomers were larger than those of control animals, and the wall appeared to be thickened. This was particularly noticeable in animals dosed with R-(-)-1-phenylethyl isothiocyanate. It is possible, therefore, that the dose of isothiocyanate employed in these experiments was too high, and the excessive concentration of the test compounds in urine may have damaged the bladder epithelium, resulting in loss of enzyme activity. A dose-response experiment will be needed to investigate this possibility.

The high inductive activity of the R-(-) enantiomers in certain tissues, compared with the isomeric S-(+) derivatives, indicates that the inductive activity of these compounds is influenced not only by substituents adjacent to the isothiocyanate group but also the stereochemistry around the isothiocyanate group. The reason for these effects is not known at present. The mechanism of induction by isothiocyanates involves the interaction with thiol groups of the Kelch-like ECH-associated protein 1 (Keap1). This interaction causes the dissociation of nuclear factor erythroid 2-related factor 2 **(**Nrf2) from Keap1, and the former translocates to the nucleus, complexes with other nuclear factors, and binds to the antioxidant response element, initiating transcription of the Phase 2 enzymes (21, 22). It is possible that the substitution and stereochemistry around this group influences the interaction of the isothiocyanate with the thiol groups of Keap1. An investigation of such interactions in cell systems *in vitro* would be of interest.

We are planning a dose-response experiment with R-(-)-1-phenylethyl isothiocyanate, in order to investigate the possibility that an excessive, and toxic, dose was employed in the present experiments. We also intend to look at ring-containing systems related to 1-cyclohexyl isothiocyanate in order to investigate the possibility that such ring structures are important for inductive activity. Such experiments will cast more light on structure-activity relationships in the inductive activity of isothiocyanates. An understanding of such relationships is important for three reasons. Firstly, it will allow prediction of inductive activity on the basis of structure, permitting the identification of particularly active substances, and by reference to the compounds contained in

Brassica vegetables, the identification of particularly active members of the Brassica family, which may have beneficial effects in humans even at low dietary intakes. Secondly, it will be possible to design isothiocyanates with high activity, which, even if synthetic rather than of natural origin, could be beneficial to individuals particularly at risk of bladder cancer, such as those with naturally low activities of Phase 2 enzymes. Thirdly, confirmation of the substitution requirements of isothiocyanates will provide valuable information on the way in which chemicals react with Keap1, and permit identification of compounds other than isothiocyanates that may be effective chemoprotective agents.

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