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EFFECT OF EMODIN ON COOKED-FOOD MUTAGEN ACTIVATION

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Abstract—The herbs *Rheum palmatum* B and *Polygonum cuspidatum* S are frequently used as laxatives and anticancer drugs in Chinese medicine. The antimutagenic activity of these herbs as well as their active component emodin was examined in *Salmonella typhimurium* TA98. The crude extracts and emodin induced a dose-dependent decrease in the mutagenicity of benzo[*a*]pyrene (B[*a*]P), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). Furthermore, emodin reduced the mutagenicity of IQ by direct inhibition of the hepatic microsomal activation and not by interaction with proximate metabolites of IQ and/or by modification of DNA repair processes in the bacterial cell.

INTRODUCTION

Emodin, a naturally-occurring anthraquinone, is present in the plant families Polygonaceae and Rhamnaceae (Brown, 1980). It is also produced by several organisms and especially by fungi (Anke *et al.*, 1980). This compound has been shown to exhibit a variety of biological effects such as inhibition of growth in the murine leukaemia virus (Kawai *et al.*, 1984), antimicrobial activity (Brown, 1980), hepatotoxicity (Keno and Saheki, 1968) and genotoxicity. In bacterial mutagenicity tests, emodin was mutagenic after metabolic activation in Salmonella strains TA97, TA102, TA1537 and TA2637, but not in TA98 (Brown and Brown, 1976; Tikkanen *et al.*, 1983). In the sister chromatid exchange assay and the hypoxanthine–guanine phosphoribosyl transferase (HGPRT) forward-mutation assay system, no genotoxicity was found either with or without metabolic activation (Bruggeman and Van der Hoeven, 1984). Emodin has also been reported to be cytotoxic in FM3A cells and to induce 6-thioguanine-resistant mutations (Morita *et al.*, 1988).

Recently, much attention has been focused on antimutagenic and anticarcinogenic constituents of the human diet. Emodin is a product of food-borne fungi and a constituent of edible and medicinal plants such as *Rheum palmatum* B and *Polygonum cuspidatum* S, which are frequently used as laxatives and anticancer drugs in Chinese medicine. In our previous report comparing 41 kinds of Chinese herbs (Lee *et al.*, 1989), extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S showed the greatest inhibitory effects on 1-nitropyrene (1NP)- and benzo[*a*]pyrene (B[*a*]P)-induced mutations. These antimutagenic effects are attributed to various polyphenols contained in the extracts such as emodin. In the present study, we investigated further the effect of the two herbs and emodin on bacterial mutagenicity induced by B[*a*]P, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). These mutagens/carcinogens have been isolated and identified from cooked proteinaceous foods (Kasai *et al.*, 1980; Lijinsky and Shubik, 1964; Sugimura *et al.*, 1977). The mutagenicity assay used *Salmonella typhimurium* TA98 with S-9 mix as the metabolic activation system.

Abbreviations: B[*a*]P = benzo[*a*]pyrene; DMSO = dimethylsulphoxide; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; Trp-P-2 = 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole.

MATERIALS AND METHODS

Chemicals and materials. Emodin, B[*a*]P, glucose-6-phosphate (G-6-P) and NADP were from Sigma Chemical Co. (St Louis, MO, USA); Trp-P-2 and IQ were obtained from Wako Pure Chemicals Co. (Japan). Extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S were prepared using the method described previously (Lee *et al.*, 1989).

Bacterial strain. S. typhimurium TA98 was a generous gift from Professor B. N. Ames, University of California (Berkeley, CA, USA). Bacteria were stored in a liquid nitrogen tank and periodically checked for their genetic markers (Maron and Ames, 1983).

Metabolic activation system. Aroclor 1254 was administered ip to male Sprague–Dawley rats (180–200 g) at a dose of 500 mg/kg body weight. The animals were starved overnight on the fourth day and killed by cervical dislocation on the fifth day. The liver was removed under sterile conditions. S-9 fractions and S-9 mix were prepared as described by Maron and Ames (1983). Protein concentrations were quantitatively determined by the method of Lowry *et al.* (1951).

Antimutagenicity assay. The method used was basically the plate-incorporation assay described by Maron and Ames (1983). To 2 ml molten top agar (45°C), were added 0.05 ml mutagen in dimethylsulphoxide (DMSO) (B[*a*]P, 100 μg/ml; IQ, 40 ng/ml;

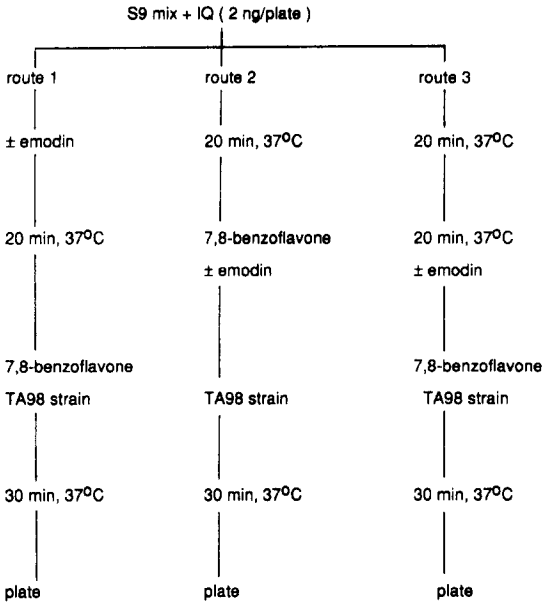

Fig. 1. Experimental protocols to study the effect of emodin on the hepatic microsomal activation of IQ.

Trp-P-2, 40 ng/ml), 0.05 ml antimutagen in DMSO, 0.1 ml overnight TA98 culture and 0.5 ml S-9 mix. The resultant mixture was gently mixed and poured onto minimal glucose agar plates. The plates were incubated at 37°C for 48 hr and the number of revertant colonies was counted. All experiments were performed twice and each assay was done in duplicate. The results were expressed as means of six plates (with standard deviations). The percentage of inhibition (PI) was calculated as described previously (Lee and Lin, 1988).

Effect of emodin on the hepatic microsomal activation of IQ. In preliminary experiments to determine optimal assay conditions, the ability of S-9 mix containing various amounts of S-9 protein to activate IQ was evaluated (Fig. 1, route 1). IQ and S-9 mix were incubated for a known incubation time in the absence of emodin. 0.1 ml of *S. typhimurium* TA98 and 0.05 ml of 7,8-benzoflavone (200 μg/ml DMSO) were then added. Incubation at 37°C was continued for another 30 min, after which top agar was added and the mixture poured onto a minimal glucose agar plate. The plates were incubated at 37°C for 48 hr and the resulting colonies counted.

The three experimental protocols used to study the effects of emodin were originally designed by Alldrick and Rowland (1988) and were adapted to our optimal assay conditions (Fig. 1). The first experiment was carried out as described above with the addition of emodin (Fig. 1, route 1). In the second experiment, the procedure was carried out with a slight modification. IQ and S-9 mix were incubated together for 20 min; emodin and 7,8-benzoflavone were then added and the mixture was incubated for another 20 min. Finally, 0.1 ml overnight-cultured bacteria was added to the reaction mixture and the assay was performed as described above (Fig. 1, route 2). In the third experiment, IQ and S-9 mix were incubated together at 37°C for 20 min. Emodin was then added and the mixture was incubated for another 20 min. Finally, 0.1 ml overnight-cultured bacteria and 0.1 ml of 7,8-benzoflavone were added to the reaction mixture and the assay was performed as described above (Fig. 1, route 3). All data were subject to two-way analysis of variance and the Student–Newman–Keuls test (Sokal and Rohlf, 1981 and 1969).

Antimutagenic effect of emodin on IQ activation. The bio-antimutagenicity test was conducted according to the pretreatment assay described by Gichner *et al.* (1987). 0.1 ml of an overnight bacterial culture, 0.05 ml IQ (40 ng/ml DMSO) and 0.45 ml of S-9 mix (containing 0.7 mg S-9 protein) were incubated in a total volume of 0.5 ml for 30 min. The incubation mixture was then washed twice with buffer by centrifugation (6000 rpm, 2°C, 10 min) to remove excess reagents. The bacteria were resuspended in the buffer to which 0.05 ml emodin was added and the liquid was incubated for 30 min in a total volume of 0.5 ml. 2 ml top agar was then added and the mixture was poured onto minimal glucose agar plates. The revertant colonies were counted after 48 hr of incubation and the data were statistically analysed by two-way analysis of variance (Sokal and Rohlf, 1981).

Toxicity analysis. The toxicity of emodin in *S. typhimurium* TA98 was tested as reported previously (Lee *et al.*, 1989; Lee and Lin, 1988).

Table 1. Antimutagenicity of extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S against B[*a*]P (5.0 μg/plate), IQ (2.0 ng/plate) and Trp-P-2 (2 ng/plate) in *S. typhimurium* TA98 with S-9 mix

		No. of revertants/plate (PI)†		
Crude extract*	μg/plate	B[*a*]P	IQ	Trp-p-2
Rheum palmatum B	125	176 ± 9 (61.4)	1021 ± 15 (23.0)	2832 ± 136 (−83.7)
	250	167 ± 8 (63.9)	315 ± 9 (78.2)	1482 ± 79 (5.0)
	500	46 ± 13 (97.2)	98 ± 7 (95.2)	483 ± 54 (70.6)
Polygonum cuspidatum S	125	186 ± 20 (58.7)	887 ± 31 (33.5)	2849 ± 69 (−84.8)
	250	162 ± 13 (65.3)	474 ± 15 (65.8)	1931 ± 55 (−24.5)
	500	93 ± 15 (84.3)	217 ± 18 (85.9)	152 ± 33 (92.4)
	0	399 ± 24	1316 ± 20	1562 ± 68
	SR	36 ± 2		

SR = spontaneous revertants

*The background revertants obtained with extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S at the amounts used were within the range of spontaneous revertants of *S. typhimurium* TA98.

†PI (percent inhibition) = 100 − (no. revertants per plate in the presence of inhibitor—spontaneous revertants)/(no. revertants per plate in the absence of inhibitor—spontaneous revertants) × 100.

Values are means ± SD.

Table 2. Determination of the toxicity of emodin in *S. typhimurium* TA98

Emodin (μg)	No. of surviving colonies/plate	Survival (%)
1.6	543 ± 13	99.1
3.1	539 ± 20	98.4
6.3	546 ± 16	99.6
12.5	518 ± 5	94.5
25.0	524 ± 11	95.6
50.0	511 ± 15	93.2
0	548 ± 22	

Values are means ± SD.

RESULTS

Table 1 shows the effects of crude extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S on the mutagenicity of B[*a*]P, IQ and Trp-P-2 in Salmonella TA98. The plant extracts showed marked dose-dependent inhibitory effects on the mutagenic potential of B[*a*]P and IQ. Both extracts exhibited a similar degree of antimutagenicity. However, their inhibitory effects on Trp-P-2 activation were less profound. The extracts were effective only at a dose higher than 500 μg per plate; at low doses, they did not inhibit Trp-P-2 mutagenicity and, on the contrary, markedly enhanced its mutagenicity. As described previously (Lee *et al.*, 1986), none of the plant extracts tested showed any toxicity to TA98 at a dose as high as 1 mg per plate.

The toxicity of emodin in TA98 was very low as indicated by the fact that the bacterial strain was not affected by an amount of emodin as high as 50.0 μg per plate (Table 2). Emodin inhibited in a comparable fashion the mutagenicity of B[*a*]P, IQ and Trp-P-2. It is interesting to note that even at a very low amount, such as 6.3 μg per plate, emodin showed a significant inhibitory effect (Table 3). Of the three mutagens studied, the highest antimutagenic effect was observed with IQ followed by Trp-P-2 and B[*a*]P.

The results of preliminary experiments are shown in Figs 2 and 3 and Table 4. It was decided to perform the assays using 0.7 mg S-9 protein in 0.5 ml of the reaction mixture, and to incubate IQ (2.0 ng/plate) and S-9 mix together for 20 min prior to adding the bacterial/7,8-benzoflavone (10.0 μg/plate) mixture.

Table 3. Antimutagenicity of emodin against B[*a*]P (5.0 μg/plate), IQ (2.0 ng/plate) and Trp-P-2 (2.0 ng/plate) in *S. typhimurium* TA98

Emodin (μg)*	No. of revertants/plate (PI)†		
	B[*a*]P	IQ	Trp-P-2
1.6	—	561 ± 50 (59.2)	1104 ± 60 (39.0)
3.1	—	345 ± 32 (76.2)	553 ± 34 (70.6)
6.3	263 ± 18 (38.0)	178 ± 20 (89.3)	339 ± 13 (82.9)
12.5	216 ± 12 (51.1)	79 ± 11 (97.0)	144 ± 5 (94.1)
25.0	177 ± 17 (62.0)	—	—
50.0	123 ± 11 (77.1)	—	—
0	399 ± 24	1316 ± 20	1785 ± 96
SR	41 ± 5		

SR = spontaneous revertants

*The background revertants of emodin at the amounts used were within the range of spontaneous revertants of *S. typhimurium* TA98.

†PI (percent inhibition) = 100 − (no. of revertants per plate in the presence of inhibitor—spontaneous revertants)/(no. of revertants per plate in the absence of inhibitor—spontaneous revertants) × 100.

Values are means ± SD.

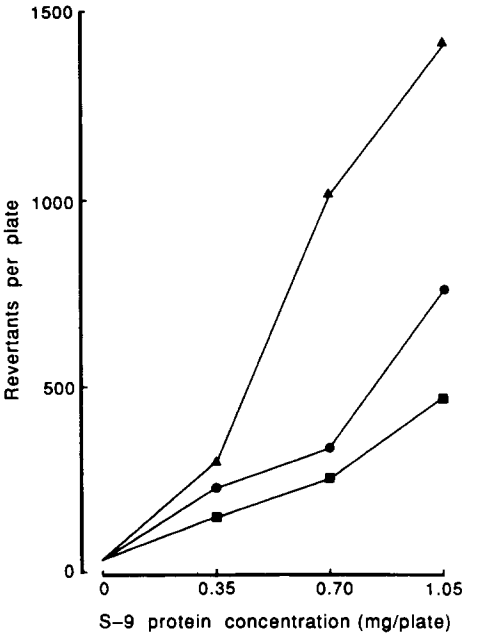

Fig. 2. Effect of increasing S-9 protein concentration on the mutagenicity of three different concentrations of IQ (■, 0.5 ng; ●, 1.0 ng; ▲, 2.0 ng). The assay mixtures were incubated at 37°C for 30 min before addition of 0.1 ml of *S. typhimurium* TA98 and 7,8-benzoflavone (10.0 μg/plate).

In order to investigate at what level emodin inhibited IQ mutagenicity (i.e. at the microsomal activation level, by direct interaction with proximate metabolites of IQ, or by modification of DNA metabolism), experiments were performed as described in Fig. 1. The results are shown in Table 5. Emodin at a dose as low as 0.5 μg per plate almost completely inhibited IQ mutagenicity at the microsomal activation level (Fig. 1, route 1). However, in the other two experiments [(i) adding emodin and 7,8-benzoflavone together (Fig. 1, route 2) to demonstrate the direct

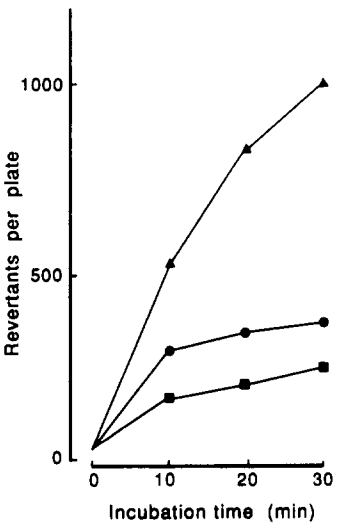

Fig. 3. Effect of incubation time between adding three different concentrations of IQ (■, 0.5 ng; ●, 1.0 ng; ▲, 2.0 ng) to 0.5 ml S-9 mix (containing 7.0 mg S-9 protein) and *S. typhimurium* TA98/7,8-benzoflavone (10.0 μg/plate).

Table 4. Effect of 7,8-benzoflavone on the metabolic activation of IQ*

7,8-Benzoflavone (μg/plate)	Revertants/plate
0	986 ± 5
5.0	247 ± 3
10.0	80 ± 2
20.0	toxic
SR	41 ± 1

SR = spontaneous revertants

*The experimental conditions were: IQ 2.0 ng/plate; 0.5 ml of S-9 mix (containing 0.7 mg S-9 protein); incubation mixture, 20 min.

Values are means ± SD.

Table 5. Effect of emodin on the hepatic microsomal activation of IQ

Emodin (μg)	No. of revertants/plate (PI)*		
	Route 1	Route 2	Route 3
0.02	966 ± 32 (26.2)	994 ± 135 (23.4)	869 ± 5 (28.5)
0.10	527 ± 67 (61.5)	964 ± 127 (25.8)	833 ± 8 (31.6)
0.50	233 ± 4 (85.1)	891 ± 55 (31.7)	813 ± 8 (33.3)
0	1293 ± 50	1283 ± 33	1196 ± 8
SR	47 ± 3		

SR = spontaneous revertants

*The background revertants at the amounts of emodin used were within the range of spontaneous revertants of *S. typhimurium* TA98.

Values are means ± SD.

Experiments are performed according to Fig. 1 and details of the procedure are described in the text. Significant differences were observed between routes 1 and 3, routes 1 and 2 and routes 2 and 3 at all emodin concentrations used ($P < 0.01$); at 0.50 μg emodin, the difference between routes 2 and 3 was not significant ($P > 0.05$). (Student–Newman–Keuls test). For each of the three routes (1, 2 and 3), highly significant differences were found between the doses of emodin used ($P < 0.001$; two-way analysis of variance).

interaction of emodin with proximate metabolites of IQ, and (ii) adding emodin and 7,8-benzoflavone separately to the pre-incubated mixture of S-9 mix and IQ to show the effects of emodin on the modification of DNA metabolism (Fig. 1, route 3)], the results did not indicate a dramatic effect of emodin on the mutagenicity of IQ. The antimutagenic effect of emodin (6.3 μg/plate) on IQ (2.0 ng/plate) was investigated . A PI value of only 20.2% was obtained (Table 6). This inhibitory effect was much lower than that of the hepatic microsomal activation. This result strongly suggests that emodin exerts its effect on the mutagenicity of IQ through direct inhibition at the hepatic microsomal activation level.

Table 6. Antimutagenic effect of emodin on IQ activation*

Emodin (μg)†	Revertants/plate	PI(%)‡
1.6	1589 ± 109	9.4§
3.2	1523 ± 82	13.3
6.3	1404 ± 64	20.2
0	1751 ± 101	
SR	33 ± 6	

SR = spontaneous revertants

*The amounts of IQ and S-9 mix used were: 2.0 ng/plate and 0.7 mg S-9 protein/plate, respectively.

†The background revertants at the amounts of emodin used were within the range of spontaneous revertants of *S. typhimurium* TA98.

‡PI (percent inhibition) = 100 − (no. of revertants per plate in the presence of inhibitor − spontaneous revertants)/(no. of revertants per plate in the absence of inhibitor − spontaneous revertants) × 100.

§Highly significant differences between doses of emodin used ($P < 0.001$; two-way analysis of variance).

Values are means ± SD.

DISCUSSION

The present investigation reports for the first time the antimutagenic activity of emodin against cooked-food mutagens in *S. typhimurium* TA98 with S-9 mix. The effects of extracts of *Rheum palmatum* B and *Polygonum cuspidatum* S on the mutagenicity of Trp-P-2 differed from those observed with B[*a*]P and IQ. The explanation for this difference still remains unknown. The two plant extracts and emodin exhibited no mutagenicity or toxicity in the bacterial strain used in our previous (Lee *et al.*, 1989) and present studies.

IQ and Trp-P-2 have been found to be mutagenic in the Ames test (Sugimura *et al.*, 1983) and carcinogenic in laboratory animal studies (Sugimura, 1985). Thus, it is important to identify dietary factors that can modify these genotoxic effects. In *in vitro* assays, a number of substances have been shown to modify the mutagenicity of these mutagens. For example, Fukahara *et al.* (1981) observed that polyphenolic compounds inhibited the formation of mutagens in albumin pyrolysates, and Tsuda *et al.* (1983) found that chlorinated tap-water reduced the mutagenicity of Trp-P-2, 2-amino-6-methyldipyrido[1,2-*a*:3′,2-*d*]-imidazole (Glu-P-1) and IQ. Alldrick *et al.* (1986) have demonstrated that many cooked-food mutagens such as IQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and Trp-P-2 can be inactivated by plant flavonoids. Melanoidin, an end-product of the Maillard reaction, has also been observed to inhibit the genotoxicity of Trp-P-1 and Trp-P-2 (Kato *et al.*, 1985). Ong *et al.* (1989) indicated that chlorophyllin showed an antimutagenic activity against mutagens in fried beef. Our results suggest that emodin is also a significant antimutagenic agent against B[*a*]P, IQ and Trp-P-2.

Huang *et al.* (1985) demonstrated that several hydroxylated anthraquinones inhibited the mutagenicity of B[*a*]P diol-epoxide by accelerating its degradation in an aqueous solution. Emodin might have a similar action on the mutagenicity of B[*a*]P.

Naturally-occurring plant polyphenols are known to be the inhibitors of the microsomal mixed-function oxidase system and particularly of cytochromes *P*-448 (Ayrton *et al.*, 1987; Steel *et al.*, 1985). *N*-hydroxylamine formation through a *P*-448 cytochrome species is a common step in the metabolic activation of IQ and Trp-P-2 (Kato and Yamazoe, 1987). The results of route 1 (Table 4) show that emodin decreases markedly the mutagenicity of IQ, presumably by inhibiting cytochrome *P*-448 activity or by interacting directly with the microsome-generated proximate mutagens to generate an inactive complex. The latter mechanism has been previously reported for the interaction between the diol-epoxide of B[*a*]P and

ellagic acid (Hayatsu *et al.*, 1988). However, it is unlikely that emodin interacts directly with proximate metabolites of IQ since our results indicate that the addition of emodin after incubation of IQ and S-9 mix failed to decrease the mutagenicity of IQ.

In conclusion, the present study demonstrates that emodin is a potent inhibitor of the mutagenicity of B[*a*]P, IQ and Trp-P-2 in *S. typhimurium* TA98. The antimutagenic effect of emodin on cooked-food mutagens involves at least two possible mechanisms of action, as mentioned above. The results of the present investigation strongly suggest that emodin inhibits the microsomal metabolism of IQ, but does not generate an inactive complex with cooked-food mutagens and/or interact with DNA repair processes in the bacterial cell. Further studies to assess the effects of emodin on cytochrome *P*-450/*P*-448 functions and to identify the ultimate metabolites of cooked-food mutagens are in progress.

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REFERENCES

Alldrick A. J. and Rowland I. R. (1988) Caffeine inhibits hepatic-microsomal activation of some dietary genotoxins. *Mutagenesis* **3,** 423–427.

Alldrick A. J., Flynn J. and Rowland I. R. (1986) Effects of plant-derived flavonoids and polyphenolic acids on the activity of mutagens from cooked food. *Mutation Research* **163,** 225–232.

Anke H., Kolthoum I., Zahner H. and Laatsch H. (1980) Metabolic products of microorganisms. The anthraquinones of the *Aspergillus glaucus* group I. Occurrence, notation, identification and antimicrobial activity. *Archives of Microbiology* **126,** 223–230.

Ayrton A. D., Lewis D. F. G., Ioannides C. and Walker R. (1987) Anthraflavic acid is a potent and specific inhibitor of cytochrome P-448 activity. *Biochimica et Biophysica Acta* **916,** 328–331.

Brown J. P. and Brown R. J. (1976) Mutagenesis by 9,10-anthraquinone derivative and related compounds in *Salmonella typhimurium*. *Mutation Research* **40,** 203–224.

Brown J. P. (1980) A review of the genetic effects of naturally occurring flavonoids, anthraquinone related compounds. *Mutation Research* **75,** 243–277.

Bruggeman I. M. and Van der Hoeven J. C. M. (1984) Lack of activity of the bacterial mutagen emodin in HGPRT and SCE assay with V79 Chinese hamster cells. *Mutation Research* **138,** 219–224.

Fukuhara Y., Yoshida D. and Goto F. (1981) Reduction of mutagenic products in the presence of polyphenols during pyrolysis of protein. *Agricultural and Biological Chemistry* **45,** 1061–1066.

Gichner T., Veleminsky J., Rapoport I. A. and Vasilieva S. V. (1987) Antimutagenic effect of *p*-aminobenzoic acid on the mutagencity of N-methyl-N′-nitro-N-nitrosoguanidine in *Salmonella typhimurium*. *Mutation Research* **192,** 95–98.

Hayatsu H., Arimoto S. and Negishi T. (1988) Dietary inhibitors of mutagenesis and carcinogenesis. *Mutation Research* **202,** 429–446.

Huang M.-T., Chang R. L., Wood A. W., Newmark H. L., Sayer J. M., Yagi H., Jerina D. M. and Conney A. H. (1985) Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by tannic acid, hydroxylated anthraquinones and hydroxylated cinnamic acid derivatives. *Carcinogenesis* **6,** 237–242.

Kasai H., Yamaizumi Z., Wakabayashi K., Nagao M., Sugimura T., Yokoyama S., Miyazawa T., Spingan N. E., Weisburger J. H. and Nishimura S. (1980) Potent novel mutagens produced by broiling fish under normal conditions. *Proceedings of the Japan Academy* **56B,** 278–383.

Kato H., Kim S. B., Hayas F. & Chuyen N. V. (1985) Desmutagenicity of melanoidins against mutagenic pyrolysates. *Agricultural and Biological Chemistry* **49,** 3093–3095.

Kato R. and Yamazoe Y. (1987) Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. *Japanese Journal of Cancer Research* **78,** 297–311.

Kawai K., Kato T., Mori H., Kitamura J. and Nozawa Y. (1984) A comparative study on cytotoxicities and biochemical properties of anthraquinone mycotoxins emodin and skyrin from *Penicillium islandicum*. *Toxicology Letters* **20,** 155–160.

Keno Y. and Saheki M. (1968) Inhibition effects of luteoskyrin, a hepatotoxic pigments of *Penicillium islandicum* Sopp. and chemically related pigment on a protozoon *Tetrahymena pyriformis*. *Japanese Journal of Experimental Medicine* **38,** 157–164.

Lee H., Chen S. C., Shiow S. J. and Lin J. Y. (1989) Effects of extracts from anticancer Chinese medicine herbs on mutagenicity of 1-nitropyrene and benzo[a]pyrene in *Salmonella typhimurium*. *Journal of the Chinese Oncology Society* **5,** 86–99.

Lee H. and Lin J. Y. (1988) Antimutagenic activity of extracts from anticancer drugs in Chinese medicine. *Mutation Research* **204,** 229–234.

Lijinsky W. and Shubik P. (1964) Benzo[a]pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science, New York* **145,** 53–55.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193,** 265–275.

Maron D. M. and Ames B. N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutation Research* **113,** 173–215.

Morita H., Umeda M., Masuda T. and Ueno Y. (1988) Cytotoxic and mutagenic effects of emodin on cultured mouse carcinoma FM3A cells. *Mutation Research* **204,** 329–332.

Ong T., Whong W. Z., Stewart J. D. and Brockman H. E. (1989) Comparative antimutagenicity of 5 compounds against 5 mutagenic complex mixtures in *Salmonella typhimurium* TA98. *Mutation Research* **222,** 19–25.

Sokal R. R. and Rohlf F. J. (1969) Single classification analysis of variance. In *Biometry: The Principle and Practice of Statistics in Biological Research*. 1st Ed. pp. 239–242. W. H. Freeman, San Francisco.

Sokal R. R. and Rohlf F. J. (1981) Two-way analysis of variance. In *Biometry: The Principle and Practice of Statistics in Biological Research*. 2nd Ed. pp. 332–344. W. H. Freeman, San Francisco.

Steel C. M., Lalies M. and Ioannides C. (1985) Inhibition of the mutagenicity of aromatic amines by the plant flavonid (+)-catechin. *Cancer Research* **45,** 3573–3577.

Sugimura T., Kawachi T., Nagao M., Yahagi T., Seino Y., Okamoto T., Shudo K., Kosuge T., Tsuji K., Wakabayashi K., Iitaka Y. and Itai A. (1977) Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. *Proceedings of the Japan Academy* **53B,** 58–61.

Sugimura T. (1985) Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. *Mutation Research* **150,** 33–41.

Sugimura T. and Sato S. (1983) Mutagens and carcinogens in foods. *Cancer Research* (Suppl.) **43,** 2415s–2421s.

Tikkanen L., Matsushima T. and Natori S. (1983) Mutagenicity of anthraquinones in Salmonella preincubation test. *Mutation Research* **116,** 297–304.

Tsuda M., Wakabayashi K., Hirayama T., Kawachi T. and Sugimura T. (1983) Inactivation of potent pyrolysate mutagens by chlorinated tap water. *Mutation Research* **119,** 27–34.