43. *Mutagenicity of Flavone Derivatives*[*]

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Bracken has long been considered to contain carcinogenic principles (Evans and Mason, 1965; Hirono *et al.*, 1970), however, isolation of carcinogenic compounds from bracken has not been successful. Overlapping of mutagens and carcinogens is now well established (McCann *et al.*, 1975; Sugimura *et al.*, 1976). We found that a flavonoid compound, kaempferol isolated from bracken was mutagenic. This finding prompted us to investigate mutagenicities of other flavone compounds, which were known to exist in various plants (Harborne *et al.*, 1975; Harborne, 1977).

Kaempferol was extracted and purified from bracken. Quercetin and fisetin were purchased from Tokyo Kasei Kogyo Co., galangin from Fulka AG., chrysin and flavone from Aldrich Chemical Co., and 3-hydroxyflavone from Eastman Kodak Co. Isoflavone derivatives, daizein, genistein and orobol were kindly provided by Dr. Tomio Takeuchi, Institute of Microbial Chemistry, Tokyo. Naringenin, a flavanone derivative, was purchased from Tokyo Kasei Kogyo Co. The purities of these compounds were confirmed by thin-layer chromatography.

Salmonella typhimurium TA98 and TA100 were used (Ames *et al.*, 1975). Test compounds were preincubated with S-9 Mix from the liver of rats which had been treated by polychlorinated biphenyl as described previously (Nagao *et al.*, 1977).

Table I shows the structures of the substances tested and their mutagenic activities in terms of number of revertants per nmole. Kaempferol, quercetin and galangin showed significantly high mutagenic activities. It is suggested from their structures that the

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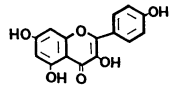
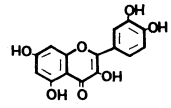
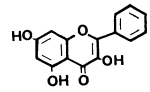
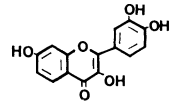
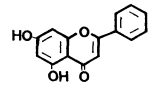
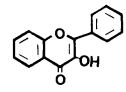
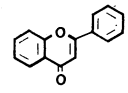
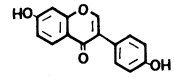
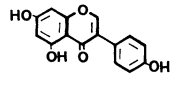
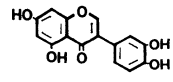
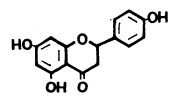
Table I. The structures and mutagenic activities of flavone derivatives

Name	Structure	His+ revertants/nmole*	
		TA98	TA100
Flavone			
Kaempferol		3.9	0.68
Quercetin		5.3	0.35
Galangin		3.8	1.6
Fisetin		0.12	0.35
Chrysin		0	0
3-Hydroxyflavone		0	0
Flavone		0	0
Isoflavone			
Daizein		0	0
Genistein		0	0
Orobol		0	0
Flavanone			
Naringenin		0	0

* Using S-9 Mix.

hydroxy groups on their phenyl moiety would not be essential in the mutagenic activity, but the 3,5,7-trihydroxyflavone structure would be important in exhibition of the mutagenic action. Fisetin which was a 3,7-dihydroxy derivative was weakly mutagenic, but chrysin, a 5,7-dihydroxy compound, did not show any mutagenic activity. 3-Hydroxyflavone and flavone were non-mutagenic. None of the three isoflavones tested was mutagenic at all. Flavanone (not listed in Table I) and 5,7,4′-trihydroxyflavanone, named naringenin, which is known to be present in fruits such as grapefruit (Dunlap and Wender, 1962) were not mutagenic at all.

Many flavonoids exist as glycosides in plants. A typical example is rutin, which is the 3-rutinoside of quercetin. Astragalin which was found in bracken is the 3-glucoside of kaempferol (Kuroyanagi *et al.*, 1974; Nakabayashi, 1955). Extensive studies on the mutagenicities of various flavones and their glycosides are now in progress in our laboratories.

The mutagenicities of kaempferol and quercetin are in the same order as those of *o*-aminoazotoluene and 4-aminobiphenyl with TA98 and in the same order as that of 3′-methyl-4-dimethylaminoazobenzene with TA100, under the standardized conditions used in our laboratories. Although quercetin and several other flavone compounds have been reported to be non-carcinogenic (DeEds, 1968), the results described here indicate the need for further carcinogenic tests on flavone derivatives. Long-term *in vivo* experiments are now in progress on the carcinogenicities of kaempferol, quercetin and rutin. Carcinogens in natural foods should be more and more studied as well as those in cooked foods, cigarette smoke, polluted air and water, etc.

References

Ames, B. N., McCann, J., and Yamasaki, E. (1975): Mutation Res., **31**, 347–364.
DeEds, F. (1968): Comprehensive Biochem., **20**, 127–171.
Dunlap, W. J., and Wender, S. H. (1962): Anal. Biochem., **4**, 110–115.
Evans, I. A., and Mason, J. (1965): Nature, **208**, 913–914.
Harborne, J. B. (1977): Biochem. System. Ecol., **5**, 7–22.
Harborne, J. B., Mabry, T. J., and Mabry, H. (1975): The Flavonoids. Chapman & Hall, London.
Hirono, I., Shibuya, C., Fushimi, K., and Haga, M. (1970): J. Natl. Cancer Inst., **45**, 179–188.
Kuroyanagi, M., Fukuoka, M., Yoshihira, K., and Natori, S. (1974): Chem. Pharm. Bull. Tokyo, **22**, 2762–2764.
McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. (1975): Proc. Natl. Acad. Sci. US., **72**, 5135–5139.
Nakabayashi, T. (1955): Bull. Agr. Chem. Soc. Japan, **19**, 104–109.
Nagao, M., Yahagi, T., Seino, Y., Sugimura, T., and Ito, N. (1977): Mutation

Res., **42**, 335–342.
Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M., and Kawachi, T. (1976): Fundamentals in Cancer Prevention (eds, P. N. Magee, *et al.*), 191–215. Univ. of Tokyo Press, Tokyo.