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Antimutagenic Activities of 24 Synthetic Flavones with the *Salmonella* Microsomal Assay

M. Laget[1], M. De Méo[1], J. C. Wallet[2], E. M. Gaydou[2], H. Guiraud[1] and G. Duménil[1]

[1]Laboratoire de Biogénotoxicologie et Mutagénèse Environnementale (EA 1784), Faculté de Pharmacie, 27 Bd Jean Moulin, 13385 Marseile cedex 5, France and [2]Laboratoire de Phytochimie, Faculté des Sciences et Techniques de Sciences et Techniques de Saint-Jérôme, 13397 Marseille cedex 20, France

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Twenty-four flavones were synthesized with various hydroxyl and/or methoxyl groups on A and B rings. Their antimutagenic properties were evaluated against benzo(a)pyrene (BaP) and a pool of mutagenic urine concentrate (U) using a modified liquid incubation method of Ames test. The tester strain was *Salmonella typhimurium* TA98+S9 Mix. The antimutagenic activities were calculated by non linear regression analysis and the doses of flavones (in nmoles) required for a 50% reduction of induced revertants with BaP and U were defined as the inhibition doses (ID_{50B} and ID_{50U} respectively). Seventeen flavones possessed significant antimutagenic activity against BaP. ID_{50B} ranged from 15.1 nmoles (F22) to 1000.6 nmoles (F13). Eighteen flavones showed significant antimutagenic activity against U. ID_{50U} ranged from 23.5 nmoles (F22) to 354.6 nmoles (F3). The 2',3',4'-trihydroxyflavone (F22, ID_{50B}=15.1 nmoles, ID_{50U}=23.5 nmoles) and the 2',3',4',7-tetrahydroxyflavone (F20, ID_{50B}=37.8 nmoles; ID_{50U}=62.3 nmoles) had antimutagenic activities similar to those of chlorophyllin (ID_{50B}=19.6 nmoles and ID_{50U}=44.2 nmoles) and were evaluated against B(a)P 7,8-dihydrodiol-9,10-epoxide. Against this last mutagen, the flavones which included three OH in B ring showed the highest activity and this property seemed independent of the substituent groups on A ring.

Key words : Antimutagens, Methoxyflavones, Hydroxflavones, Ames test

INTRODUCTION

Flavonoids belong to a large group of natural products which can be found in edible foods. More than 2,000 substances are known and few studies have assessed their biological properties.

Natural flavonoids exhibit the basic structure of 2-phenyl-benzo(α)pyrene or flavone nucleus including two benzene rings (A and B) linked through a heterocyclic pyrane C ring. Most of the derivatives are hydroxylated, methoxylated and/or glycosylated.

Flavonoids have been shown to present a variety of biochemical and pharmacological effects including antiinflammatory and antiallergic effects (Middleton, 1992), linking affinity to biopolymers, ability to interfere with redox reactions and free-radical scavenging (Havsteen, 1983; Sichel, 1991; Cotelle *et al.*, 1992).

The inhibition of the mutagenic activity of polycyclic aromatic hydrocarbons by phenolic plant flavonoids has been reported (Huang *et al.*, 1983; Francis *et al.*, 1989). Wall *et al.* (1988) and Choi *et al.* (1994) have also found antimutagenic flavonoids against aflatoxin B_1 (AFB_1).

These compounds have been also shown to reduce effects of clastogenic activity of carcinogens which induce sperm abnormality (Raj *et al.*, 1984) and they inhibit skin tumour promotion (Konoshima *et al.*, 1989), they inhibit also carcinogen-induced tumours in rats and in mice (Deschner *et al.*, 1991). Several reports have indicated flavonoids to be anticarcinogenic against various carcinogens: benzo(a)pyrene (BaP) (Wattenberg *et al.*, 1970; Shah *et al.*, 1986), aflatoxin (Nixon *et al.*, 1984; Chang *et al.*, 1985; Francis *et al.*, 1989), 2-aminoanthracene and N-methyl-n-nitro-N-nitrosoguanidine (Birt *et al.*, 1986).

The aim of this work was to study new flavonoids with unusual oxygenation patterns for natural compounds. We report their antimutagenic properties and an evaluation of their structure-activity relationships. The flavonoids group which was investigated was composed of polyhydroxy and/or polymethoxy flavones sterically hindered around the bond linking the γ-benzopyrone portion to the phenyl ring.

Twenty-four flavones were synthesized. However, 2'-hydroxy (Bouillant *et al.*, 1971), 2'-methoxy (Freeman *et al.*, 1981) and 5-hydroxy-2'-methoxy-

Correspondence to: G. Duménil, Laboratoire de Biogénotoxicologie et Mutagénèse Environnementale (EA 1784), Faculté de Pharmacie, 27 Bd Jean Moulin, 13385 Marseille cedex 5, France.

flavones (Wollenweber and Mann, 1986) are naturally occurring flavones. Their antimutagenic and toxic properties were assessed by salmonella microsomal assay. Their antimutagenic effects were evaluated against (BaP) and a complex mutageinc mixture (pool of urinary mutagens concentrate from smokers=U). Antimutagenic properties of some of them were also evaluated against B(a)P 7,8-diOH diol-9,10-epoxide (BPDE).

MATERIALS AND METHODS

1-Chemicals

Benzo(a)pyrene (BaP), dimethylsulfoxide (DMSO, Spectro grade) and chlorophyllin were purchased from Sigma (St Louis, MO, USA). Aroclor 1254 was from Analabs (North Heaven, CT, USA). Benzo(a) pyrene 7,8-dihydrodiol-9,10-epoxide (BPDE) was from MRI (Kansas City, Missouri).

Compounds synthesis

Compounds were prepared by addition of excess thionyl chloride to the corresponding methoxylated benzoic acid. After removal of unreacted thionyl chloride, pyridine and *o*-hydroxyacetophenone were added according to this procedure as described by Gaydou and Bianchini (1978).

Fully methoxylated compounds were obtained by boiling the hydroxylated derivatives in acetone with an excess of dimethylsulfate and sodium carbonate, to reflux during 24 hours. Fully hydroxylated compounds were obtained by demethylation with aluminum chloride in benzene. Two successive crystallisations in ethanol gave the pure products. Compounds were characterized by their melting points and their proton and carbon nuclear magnetic resonance spectra. The purities of the compounds were confirmed by thin layer chromatography. For some of them, X-ray structures have been described.

The chemical structure of the 24 flavones and the molecular weights are included in Table 1.

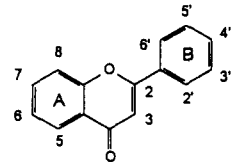
All compounds were dissolved in DMSO at various concentrations:

C=10 mg/ml (F12, F14, F16, F17, F20, F21, F22 and F24) C=20 mg/ml for the remaining compounds.

Smoker's urine concentrate (U)

A 24 h urine sample from a male smoker (40 cigarettes per day) was collected. The extraction and concentration of urinary mutagens were carried out by the absorption technique on Amberlite XAD-2 resin as described by Yamasaki and Ames (1977). The concentrated urine sample was fractionated and

Table I. Antimutagenic activity of the 24 derivatives of flavones



Basic structure of the flavone

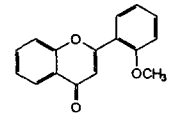
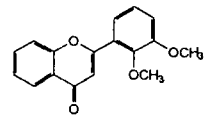
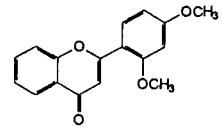
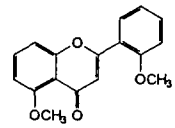
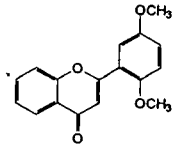
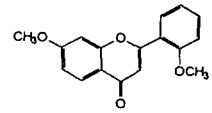
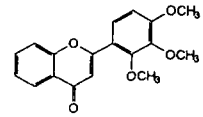
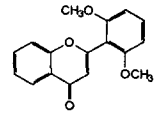
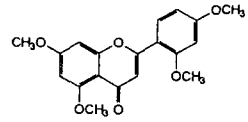
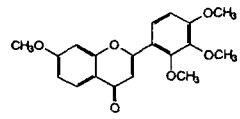
N°	FORMULA	MW	ID_{50B}	ID_{50U}
F1		252	93.2 *396.8*	52.8 *158.7*
F2		282	141.0 *141.8*	210.7 *354.6*
F3		282	134.9 *354.6*	276.6 *354.6*
F4		282	178.6 *354.6*	185.6 *354.6*
F5		282	93.2 *283.6*	133.5 *212.7*
F6		282	395.9 *354.6*	148.7 *141.8*
F7		312	91.0 *320.5*	316.0 *320.5*
F8		282	NS	NS
F9		342	190.0 *292.3*	172.2 *233.9*
F10		342	NS	396.6 *292.3*

Table I. Continued

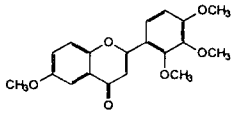
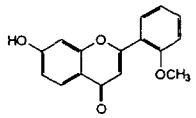
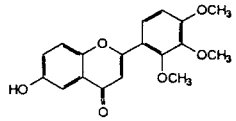
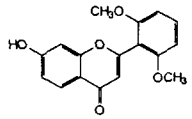
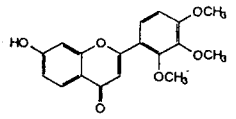
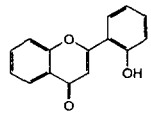
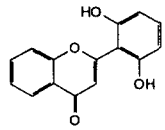
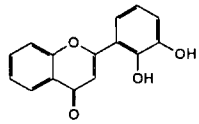
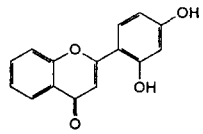
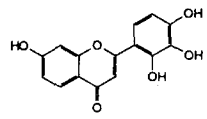
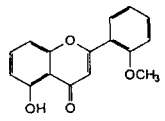
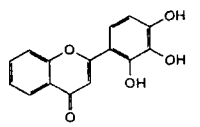
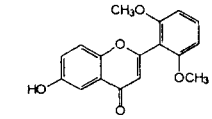
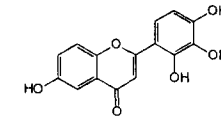
N°	FORMULA	MW	ID_{50B}	ID_{50U}
F11		342	NS	243.0 *292.3*
F12		268	NS	45.2 *149.2*
F13		328	1000.6 *121.9*	NS
F14		298	NS	NS
F15		328	364.0 *121.9*	NS
F16		238	192.8 *210.0*	165.3 *168.0*
F17		254	734.7 *78.7*	247.8 *196.8*
F18		254	264.5 *393.7*	154.0 *314.9*
F19		254	285.7 *393.7*	198.6 *157.4*
F20		286	37.8 *104.8*	62.3 *104.8*
F21		268	105.5 *149.2*	52.3 *149.2*
F22		270	15.1 *111.1*	23.5 *111.1*

Table I. Continued

N°	FORMULA	MW	ID_{50B}	ID_{50U}
F23		298	NS	NT
F24		286	NT	NT
CHL	CHLOROPHYLLIN	722	196.6 *69.3*	44.2 *69.3*

All the compounds were assayed by a modified version of Ames test using *Salmonella typhimurium* strain TA 98 (De Mé o *et al.*, 1988). The molecules are listed by their reference number (column 1). Their chemical formula and their molecular weight are indicated in columns 2 and 3, respectively. The inhibition doses for a 50% decrease of the mutagenic activity of the benzo[a]pyrene (ID_{50B}) and a mutagenic urine concentrate (ID_{50U}) is given in columns 4 and 5. The highest significant doses (nmoles/plate) of the tested flavones are in italics. These doses represent the highest ones used for the non linear regression analysis. The complete details of the technique and the calculations were described in the Materials and Methods section.
NS: not significant; the probability of the model was >0.05
NT: not tested

kept at -80°C and all the assays were performed within 2 weeks.

2-Mutagenicity and antimutagenicity assays

Tester strains: *Salmonella typhimurium* TA 97a, TA 98, TA 100 and TA 102 strains were gifts from Prof. B.N. Ames (Berkeley, CA, USA). They were stored at -80°C and regularly checked for their genetic markers (Maron and Ames, 1983).

Activation mixture: The metabolizing system: S9 Mix was prepared using Aroclor 1254 induced rats as previously described by Ames *et al.* (1975). The concentration of proteins in the S9 fraction was 31.0 mg/ml as determined by the technique of Lowry *et al.* (1951). The S9 Mix was a mixture of 4% S9 and a solution of cofactors (NADPH generating system; Maron and Ames, 1983).

Assay procedure: All samples were assessed for toxicity, mutagenicity and antimutagenicity by Ames test (Maron and Ames, 1983). Preliminary screening was performed using the semi-quantitative technique (the spot test) as previously described (Maron and Ames, 1983) with the tester strains ±S9 Mix. The tested dose was 200 µg. Following the preliminary screening all the samples were assessed for mutagenicity and antimutagenicity by the quantitative technique using a modified version of the liquid incubation

method (De Méo *et al.*, 1988, 1995). Briefly, *Salmonella* tester strain was grown overnight in Oxoid Nutrient broth n°2 with gentle shaking (final cell concentration $0.5\text{-}1\times10^9$ cells/ml). After this incubation period, the following ingredients were added to 12× 75 mm sterile polystyrene tubes on ice : 0.1 ml of S9 Mix (4% of S9), various doses of the tested mutagens not exceeding 5 µl, various doses of antimutagens or 0.1 M phosphate buffer (pH 7.4) not exceeding 5 µl and 0.1 ml of the overnight culture. The mixture was incubated for 60 min at 37°C with rapid shaking. After the contact period the tubes were placed on ice, taken out one at a time and a volume of 2 ml of molten top agar was added. The combined mixture was poured onto Vogel-Bonner salt minimal agar plates. The seeded plates were incubated for 48 h at 37°C in the dark. Spontaneous and induced revertants per plate were determined for each dose with a laser bacteria colony counter (Model 500A, Interscience). During this study, the spontaneous frequency of tester strain TA98+S9 Mix was 48±23 (n=8), the number of induced revertants with 4 nmoles of BaP was 780± 218 (n=8), with 0.0165 nmoles of BPDE was 341±3 (n=2) and the number of induced revertants with 2 ml equivalent of urine was 330±85 (n=8).

Determination of antimutagenic activity

The antimutagenic activities of the molecules were calculated by non linear regression analysis using the model:

$$Rev_d = Rev_0 * e^{(-a*d)}$$

where Rev_d=number of revertants/plate with the reference mutagen and the antimutagen at the tested dose d.

Rev_0=number of induced revertants/plate with reference mutagen
a=coefficient calculated by regression analysis
d=dose of the tested antimutagen (nmoles/plate).

The probability of the model was calculated by analysis of variance and was $p \leq 0.05$.

The doses of flavones required to reduce the induced revertants with BaP, U and BPDE by 50% were defined as the inhibition doses (ID_{50B} and ID_{50U} respectively and ID_{50DE}) and were calculated as follows:

$$ID_{50}(\text{nmoles}) = \frac{0.693}{a}$$

RESULTS AND DISCUSSION

Twenty-four synthetic flavones were tested for toxic, mutagenic and antimutagenic activities against BaP and U by Ames test. BaP was selected as a pure compound that requires metabolic activation to exert mutagenic activity. Highly reactive electrophilic metabolites such as 7,8- dihydrodio-9,10-epoxide bind to proteins and nucleic acids and induce mutagenic effects (Horikawa *et al.*, 1994). Thus, the antimutagenic effect with respect to BaP involves the inactivation of either cytochrome-dependent monooxygenases or the metabolites.

Smoker's urine is a complex mixture of mutagens. Putzrath *et al.* (1981) have detected hydrophobic molecules (HAP, aldehydes and nitrosamines) using HPLC technique. Connors *et al.* (1983) have identified 2-naphthylamine in the urine of a heavy smoker. Peluso *et al.* (1991) have detected 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) in the urine of a brown tobacco smoker. PhIP is a heterocyclic amine that is found in broiled fish and meats.

Toxic and mutagenic activites

The determination of the toxic and mutagenic activities was carried out by the modified liquid incubation assay (De Méo *et al.*, 1988, 1995) using tester strains TA97a, TA98, TA100, and TA102 with and without S9 Mix. No mutagenic or toxic compound was detected at the concentrations ranging from 10 mg/ml to 20 mg/ml.

This result was consistent with data published by Brown (1980) who had determined basic structural requirements which appear essential for mutagenic activity is the presence of the 3-OH group. This 3-OH group defines the flavonol structure.

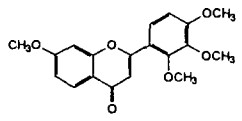
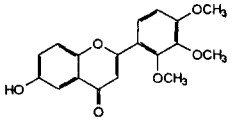
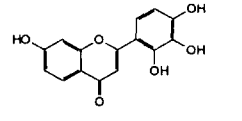
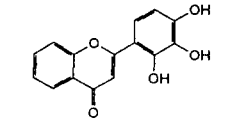
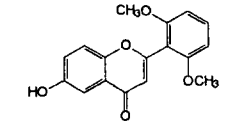
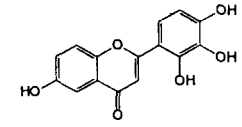
The 5-OH group also appears as an important structural determinant for intrinsic mutagenicity of the molecules. Mutagenic activity of flavones (related to norwogonin) has been shown to require a hydroxyl or methoxyl groups at position 8 (McGregor, 1986). These molecules were activated by cytosolic factors from mammalian liver. Furthermore, the response of strain TA98 was weaker than the response of strain TA100. However, mutagenic activity of flavones of this type was not dependent on a free 3-hydroxyl group and hydroxyl groups on B ring were apparently not involved in their activation (McGregor, 1986). In the present study, no molecules were substituted at 3-position.

Antimutagenic activities

Antimutagenic activities of the new flavones were also determined by the modified version of Ames test and were characterized by the determination of the ID_{50} for BaP and U and sometimes for BPDE. Assays were performed with TA98 tester strain with S9 Mix. Mutagenic compounds were BaP (1 µg) and smokers'-urine (U: 8 µl i.e. 2.0 ml equivalent) and BPDE (5 ng).

Complete data on the antimutagenic activity of the

Table II. Antimutagenic activity of some flavones against B(a)P, and BPDE

N°	FORMULA	MW	ID_{50B}	ID_{50diol}
F10		342	NS *146.0*	NS *146.0*
F13		328	1000.6 *121.9*	49.6 *152.3*
F20		286	37.8 *104.8*	17.1 *86.5*
F22		270	15.1 *111.1*	20.7 *111.1*
F23		298	NS	NS
24		286	NT	16.3 69.0
CHL	CHLOROPHYLLIN	722	19.6 69.3	8.4 69.3

All the compounds were assayed by a modified version of Ames test using *salmonella typhimurium* strain TA 98 (De Mé o *et al.*, 1988, 1995). The molecules are listed by their reference number (column 1). Their chemical formula and their molecular weight are indicated in column 2 and 3, respectively. Their inhibition doses for a 50% decrease of the mutagenic activity of benzo[a]pyrene (ID_{50B}) and benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide (ID_{50diol}) are given in columns 4 and 5. The highest significant doses (nmoles/plate) of the tested flavones are in italics. These doses represent the highest ones used for the non linear regression analysis. The complete details of the technique and the calculations were described in Materials and Methods section.
NS: not significant; the probability of the model was >0.05
NT: not tested

flavones against BaP and U are included in Table 1 and for four molecules against BPDE in Table 2. A typical dose-response curve against BaP is shown in Fig. 1 for the compound F7.

Methoxyl substitutions on B ring

Among the flavones methoxylated on B ring, the 2'-

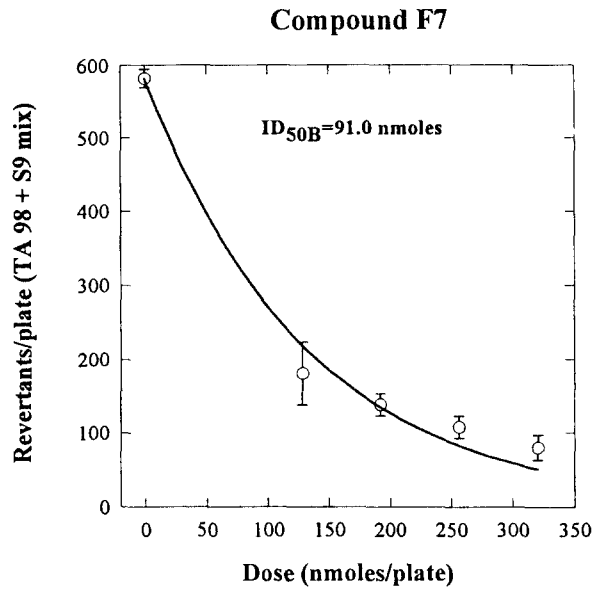


Fig. 1. Effect of flavone F7 on the mutagenic activity of BaP with strain of *Salmonella typhimurium* TA 98. The intrinsic mutagenicity of 4 nmoles of BaP in the absence of added flavone was 582±9 histidine revertants per plate and the background mutation rate was 65±3 for this series of experiments. All data represent the mean of triplicate assays. The antimutagenic activity was calculated by non linear regression analysis. The inhibition dose (ID_{50B}) was defined as the dose (nmoles) that reduced the number of induced revertants by 50% and was determined from the non linear equation.

methoxflavone (F1) was found to be the most antimutagenic molecule against the two reference mutagens (ID_{50B}=93.2 nmoles and ID_{50u}=52.8 nmoles). Addition of a second methoxyl group o this ring at 3', 4' and 6'-position decreased antimutagenic activity. However the flavone F5 with a second methoxyl group in 5' position was the most active molecule of this group (ID_{50B}=93.2 nmoles and ID_{50u}=133.5 nmoles).

The 2',6'-dimethoxyflavone (F8) was not antimutagenic against BaP and U. A third methoxyl group giving the 2',3',4'-trimthoxyflavone (F7) enhanced the antimutagenic activity against BaP (ID_{50B}=91 nmoles) but decreased it against U (ID_{50u}=316.0 nmoles).

Methoxyl and/or hydroxyl substitutions on A ring

When ID_{50B} was considered, substitution by methoxyl or hydroxyl group on A ring did not remove the antimutagenic properties except for F10, F 11 and F12. Methylation or hydroxylation on position 5 maintained the antimutagenic activity (F4, F21) which was decreased by substitution on position 6 (F 13) or 7 (F6, F15).

When ID_{50U} was considered, the position of the hy-

droxyl or methoxyl group on ring A did not modify the activity except in F13 and F15.

Hydroxyl substitutions on B ring

Hydroxylation on B ring modulated the antimutagenic activities of the flavones. The monohydroxylated compound (F16) showed antimutagenic properties (ID_{50B}=192.8 nmoles and ID_{50U}=165.3 nmoles). The addition of a second OH in a different position on B ring did not improve this activity (F17, F18, F19). However, the maximal activities were obtained with the 2',3',4'-trihydroxflavone (F22) which has been found to be the most antimutagenic compound of the series with a ID_{50B}=15.1 nmoles and ID_{50U}=23.5 nmoles. Theses values remained below those of chlorophyllin. This activity remained against BaP and U with addition of a hydroxyl in position 7 of A ring (F20) with ID_{50B}=37.8 nmoles and ID_{50U}=62.3 nmoles.

The substitution of a hydroxyl group for a methoxyl group on the 2' position increased the antimutagenic activity (F16 and F1). An additional methoxyl group led to increase antimutagenic activity against BaP and decreased activity against U (F18 and F2; F19 and F3). Finally, trimethoxylated flavone on B ring (F7) had lower antimutagenic activites than the trihydroxylated counterpart (F22) against the two reference mutagens.

Few examples of antimutagenic flavonoids have been reported in the literature (Birt *et al.*, 1986; Wall, 1988). The polyhydroxyflavonoid structure was generally highly active in inhibiting the molecular raectivity of BaP (Birt *et al.*, 1986) and AFB_1 (Wattenberg *et al.*, 1970).

Several other types of flavonoids have been also shown to be antimutagenic (Torigoe *et al.*, 1983) and antimutagenic properties of these molecules of these molecules can be influenced by the nature of the reference mutagen (Wall *et al.*, 1988; Waters *et al.*, 1990).

Glycosylation of flavonoids decreased antimutagenic activity against AFB_1. Methylation of a parent flavonoid reduced this activity (Wattenberg *et al.*, 1970).

A study of the structure-activity relationship of the flavonoids has shown that hydroxyl group on B ring are important for antimutagenic activity. Saturation of the 2,3-double bond or lack of the 4'-OH group of the phenyl substituent had been found to decrease antimutagenic activity (Huang *et al.*, 1983).

The inhibition effect of hydroxyflavonoids on the mutagenicity of BaP may result from a specitfic inhibitory effect on the monooxygenase system which metabolizes the hydrocarbons to ultimate mutagens and/or by a direct interaction of the flavonoid with the mutagenic metabolites. An inhibitory effect of several hydroxyflavonoids on microsomal monooxygenase systems has been reported for the reduced cytochrome P-450. The structural feature of flavonoids required for the inhibition of BaP hydroxylation has been shown to be hydroxyl groups (Buening *et al.*, 1981).

The intrinsic mechanism underlying this behaviour has not yet been clearly elucidated and several hypotheses concerning molecular mechanism of the action have been proposed in the literature (Pathak *et al.*, 1991).

In order to understand inhibitory effects, inhibition of the monooxygenase system or a direct interaction with the BaP-diol epoxide, several flavones from Table 1 were tested with the ultimate mutagenic and carcinogenic metabolite of BaP: BaP 7,8-dihydrodiol-9,10-epoxide.

Flavones selected were F10 (with no significant antimutagenic properties), F13 (withe a high ID_{50B}) and the two most reactive flavones F20 and F22. The results are presented in Table 2.

The fully methoxylated flavone F10 was inactive. A higher antimutagenic activity against BPDE was observed for F13. F20 was twic more effective potent inhibitor of the mutagenicity of BaP-diol epoxide than of BaP. F22 had about the same activity against BaP and diol epoxide. They produced dose-dependent inhibition of the mutagenic activity of BaP diol epoxide in strain TA98.

Since we observed a much better response for F13, we thought that an hydroxyl group in position 6 would improve the activity against BaP diol epoxide. Two additional flavones were included in this study: F23 and F24. No significant increase of the antimutagenic activity was noted as compare to F8 and F22. Thus, the most active flavones were those with a pyrogallol moiety on B ring. We have reported the antimutagenic activity of flavone sterically hindered around the bond linking the γ-benzopyrone portion and the phenyl ring. The most active flavones were the 2',3',4'-trihydroxyfavones. The antimutagenic activity seemed independent of the type of substituent on the A ring. However, Choi *et al.* (1994) have found that the OH groups on the 5 and 7 positions seemed to be essential for the antimutagenicity of the flavonoids against AFB_1.

In this study, the flavones tested had structural similarities with 3',4',5'-trihydroxyflavonols (Huang et al., 1983) and ellagic acid (Sayer *et al.*, 1982) which are known to react with BaP diol epoxide.

These observations indicate that the inhibitory effects of the hydroxylated flavonoids on the mutagenicity of polycyclic aromatic hydrocarbons may result from their direct interaction with the ultimate mutagenic metabolite diol epoxide instead of a direct

effect on the cells. These 2',3',4'-trihydroxyflavones were good interceptor of BaP diol epoxide and they could inhibit the mutagenic of BaP.

However our results show that an inhibition of the monooxygenase system that metabolizes the BaP cannot be excluded. Further experiments are required to confirm the mechanism.

REFERENCES CITED

Ames, B.N., Mc Cann, J. and Yamasaki, E., Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian microsome mutagenicity test. *Mutat. Res., 31, 347-364 (1975).*

Ames, B.N., Dietary carcinogens. *Science*, 221, 1256-1263 (1983).

Birt, D.F., Walker, B., Tibbels, M.G. and Bresnick, E., Antimutagenesis and antipromotion by apigenin, robinetin and indole-3-carbinol. *Carcinogenesis*, 7, 959-963 (1986).

Bouillant M.L., Wollenweber E. and Chopin J., Demonstration of 2'-hydroxy and 2,5'-dihydroxyflavones in the primrose *Primula florindae*. *C. R. Acad. Sci.*, Paris, 273: 1629-1631 (1971).

Brown, J.P., A review of the genetic effects of naturally occuring flavonoids, anthraquinones and related compounds. *Mutat. Res.*, 75, 243-277 (1980).

Buening, M. K., Chang, R. L., Huang, M. T., Fortner, J. G., Wood, A. W. and Conney, A. H., Activation and inhibition of BaP and AFB_1 metabolism in human liver microsome by naturally occuring flavonoids. *Cancer Res.*, 41, 67-72 (1981).

Chang, R. L.,. Huang, H. T., Wood, A. W., Wong, C. Q., Newmark, H. L., Yagi, H., Sayer, J.M., Jerina, D. M. and conney, A. H., Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of BaP and (±) 7β, 8α dihydro-9α, 10α-epoxy-7,8,9, 10-tetrahydrobenzo(a)pyrene on mouse skin and in the newborn mouse. *Carcinogenesis*, 6, 1127-1131 (1985).

Choi, J.S., Park, K.Y., Moon, S.H., Rhee, S. H. and Young, H. S., Antimautagenic effect of plant flavonoids in *Salmonella* Assay system. *Arch. Pharm. Res.* 2, 71-75 (1994).

Connors, T. H., Ramanujam, V. M. S., Ward Jr, J. B. and Legator, M. S., The identification and characterization of a urine mutagen resulting from cigarette smoke. *Mutat. Res.*, 113, 161-172 (1983).

Cotelle, N., Bernier, J. L., Hénichart, J. P., Catteau, J. P., Gaydou, E. M., and Wallet, J. C., Scavenger and antioxidant properties of ten synthetic flavones. *Free Radical Biol. Med.* 13, 211-219 (1992).

De Méo, M. P., Miribel, V., Botta, A., Laget, M. and Duménil G., Applicability of the SOS Chromotest to detect urinary mutagens caused by smoking. *Mutagenesis*, 3, 277-283 (1988).

De Méo, M., Laget, M., Di Giorgio, C., Guiraud, H., Botta, A., Castegnaro, M. and Duménil, G., Optimisantion of the *Salmonella*/mammalian microsome assay for urine mutagenesis by experimental designs. *Mutat. Res.*, (1995) (in press).

Freeman, P. W., Mjurphy, S. T., Nemorin, J. E. and Taylor W. C., The constituents of Australian Pimelea species. II. The isolation of unusual flavones from *P. simplex* and *P. decora*. *Aust. J. Chem.*, 34, 1779-1784 (1981).

Gaydou E.M. et Bianchini J.P., Etudes de composes flavoniques. I. Syntheses et propriétés (UV, RMN du ^{13}C) de quelques flavones. *Bull. Soc, Chim.* Part II, 43-47 (1978).

Havsteen, B., Flavonoids: a class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, 32, 1141-1148 (1983).

Horikawa, K., Morhi, T., Tanaka, Y. and Tokiwa, H., Moderate inhibition of mutagenicity of benzo[a] pyrene, 1,6-dinitropyrene and 3,9-dinitrofluoranthrene by chinese medecinal herbs. Mutagenesis, 9, 523-526 (1994).

Huang, M. T., wood, A. W., Newmark, H. L., Sayer, J. M., Yagi, H., Jerina, D. M. and Conney, A. H., Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids. *Carcinogenesis* 4, 1631-1637 (1983).

Konoshima, T., Takasaki, M., Kosuka, M., Inada, A., Nakanishi, T., Tokuda, H. and matsumoto, T., Studies on inhibitors of skin tumor promotion. Inhibitory effects of flavonoids on Epstein-Barr virus activation. *Shoyakugaku Zasshi*, 43, 135-141 (1989).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurements with the folin-phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

McGregor, J. T., Mutagenic and carcinogenic effects of flavonoids. In: Plant Flavonoids in Biology abd Medecine: biochemical, pharmacological and structure activity relationships. Alan R. Liss, Inc, 411-424 (1986).

Maron, D. M. and Ames, B. N., Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, 113, 173-215 (1983).

Nixon, J. E., Hendricks, J. D., Pawlowski, N. E. Pereira, C. B., Sinnhuber, R. O., and Bailey, G. S., Inhibition of aflatoxin B1 carcinogenesis in rainbow trout by flavone and indole compounds. *Carcinogenesis*, 5, 615-619 (1984).

Peluso, M., Castegnaro, M., Malaveille, C., Friesen, M., Garenne, L., Hautefeuille, A., Vineis, P., Kadlubar, F. and Bartsch, H., ^{32}P-postlabelling analysis of urinary mutagens from smokers of black tobacco implicates 2-amino-1-methyl-6-phenylimidaxo[4,5-

b]pyridine (PhIP) as a major DNA-damaging agent. *Carcinogenesis*, 12, 713-717 (1991).

Putzrath, R. M., Langley, D. and Eisenstadt, E., Analysis of mutagenic activity in cigarette smoker'surine by high performance liquid chromatography. *Mutat. Res.*, 85, 97-108 (1981).

Raj, A. S. and Morris, K., Inhibitory effect of α-naphtoflavone and β-naphtoflavone on 7,12-DMBA induced anomalies in germ cells assessed by sperm abnormality assay. *Mutat. Res.*, 136, 81-84 (1984).

Sayer, J. M., Yagi, H., Wood, A. W., Conney, A. H. and Jerina, D. M., Extremely facile reaction between the ultimate carcinogen benzo[a]pyrene-7, 8-diol-9,10-epoxide and ellagic acid. *J. Am. Chem. Soc.*, 104, 5562-5564 (1982).

Shah, G.M. and Bhattacharya, R. K., Modulation by plant flavonoids and reated phenolics of microsome catalysed adduct formation between benzo(a)pyrene and DNA. *Chem. Biol. Interactions*, 56, 1-15 (1986).

Sichel, G., Corsaro, C., Scalia, M., DiBilio, A. J. and Bonomo, R. P., *In vitro* scavenger activity of some flavonoids and melanins against O_2-. *Free Rad. Biol. Med.*, 11, 1-8 (1991).

Torigoe, T., Arisawa, M., Itoh, S., Fujiu, M. and Maruyama, H. B., Antimutagenic chalcones: antagonizing the mutagenicity of BaP on *Salmonella typhimurium*. *Biochem. Biophys. Res. Commun.* 112, 833-842 (1983).

Wattenberg, L. W. and Leong, J. L., Inhibition of carcinogenic action of benzo(a)pyrene by flavones. *Cancer Res.*, 30, 1922-1925 (1970).

Wall, M. E., Wani, M. C., Manikumar, G., Abraham, P., Taylor, H., Hughes, T. J., Warner, J. and McGivney, R., Plant Antimutagenic Agents, 2. Flavonoids. *J. Natural Products*, 51, 1084-1091 (1988).

Waters, M. D., Bradly, A. L., Stack, H. F. and Brockman, H. E., Antimutagenicity profiles for some model compounds. *Mutat. Res.*, 238, 57-85 (1990).

Wollenweber E. and Mann K., New flavonoids from *Primula farinose* exdates. *Biochem. Physiol. Pflanz.* 181, 667-671 (1986).

Wood, A. W., Huang, M.T., Chang, R. L., Newmark, H. L,., Lehr, R. E. Yagi, H., Sayer, J. M., Jerina, D. M. and Conney, A. H., Inhibition of the mutagenicity of bay-region diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: exceptional activity of ellagic acid. *Proc. Natl. Acad. Sci.*, 79, 5513-5517 (1982).

Yamasaki, E. and Ames, B. N., Concentration of mutagens from urine by absorption with the non polar resin XAD-2: cigarette smokers have mutagenic urines. *Proc. Natl. Acad. Sci.* USA, 74, 3555-3558 (1977).