Gallon, J. R., Kurz, W. G. W. & LaRue, T. A. (1972) *Can. J. Microbiol.* **18**, 327–332
Gorham, P. R., McLachlan, J. S., Hamer, V. T. & Kin, W. K. (1964) *Verh. Int. Ver. Theor. Angew. Limnol.* **15**, 790–804
Janiszowska, W. & Pennock, J. F. (1976) *Vitam. Horm. (N.Y.)* **34**, 77–107
Kratz, W. A. & Myers, J. (1955) *Am. J. Bot.* **42**, 282–288
Mereschowsky, C. (1905) *Biol. Zfl.* **25**, 593–612
Newton, R. P. & Pennock, J. F. (1971*a*) *Phytochemistry* **10**, 2323–2328
Newton, R. P. & Pennock, J. F. (1971*b*) *Biochem. J.* **124**, 21P
Powls, R. & Redfearn, E. R. (1967) *Biochem. J.* **104**, 24C
Sagan, L. (1967) *J. Theor. Biol.* **14**, 225–243
Schultz, G., Huchzermeyer, Y., Reupke, B. & Bickel, H. (1976) *Phytochemistry* **15**, 1383–1386
Stewart, W. D. P., Haystead, A. & Pearson, H. W. (1969) *Nature (London)* **224**, 226–228
Whistance, G. R. & Threlfall, D. R. (1971) *Phytochemistry* **10**, 1533–1539

Frameshift Mutagenicity of Certain Naturally Occurring Phenolic Compounds in the '*Salmonella*/Microsome' Test: Activation of Anthraquinone and Flavonol Glycosides by Gut Bacterial Enzymes

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The flavonoids are one of the more numerous groups of natural products. About 2000 substances have been described (Harborne *et al.*, 1975) and as a group the flavonoids are universally distributed among vascular plants. Similarly, the 9,10-anthraquinones represent the largest group of natural quinones and most of these materials from plants and fungi are also phenolic (Mathis, 1966; Thomson, 1971). Naturally occurring and synthetic anthraquinones have been used as colorants in foods, drugs, cosmetics, hair dyes and textiles. Various plant anthraquinone glycosides, particularly those from *Cassia*, *Rhamnus* and *Rheum* spp., traditionally have found use in purgative preparations.

Our interest in developing non-absorbable synthetic food additives with a high order of chemical and biological stability has led us to genetic screening of a relatively large number of compounds from both of these groups: anthraquinones and their close relatives as colorants and certain flavonoids as non-nutritive sweeteners. Results reported here were obtained with the *Salmonella typhimurium*/microsome test as described by Ames *et al.* (1975). Manipulation of cultures, preparation of crude microsomal fraction (S9) from Aroclor 1254-induced rats and criteria used in scoring results have been described in detail previously (Brown & Brown, 1976). Enzymic extracts of rat caecal bacteria were prepared the day of use by sonic treatment (30W, 10min) of a freshly prepared suspension of caecal contents in one-quarter-strength Krebs–Ringer 0.25M-phosphate buffer, pH7.4 (1g fresh wt./10ml). After sedimentation of cellular debris at 18000*g* for 20min at 4°C, the supernatant was removed and sterilized by filtration.

A high percentage of anthraquinones exhibited mutagenicity for strains TA1537 (*hisC*3076), TA1538 and TA98 (both *hisD*3052), which are particularly sensitive to frameshifting mutagens (Table 1). Activity with strain TA100 (*hisG*46) was seen in a few cases. Although the *hisG*46 mutation is specific for reversion by base-pair substitution, the presence in the TA100 strain of a plasmid (pKM101) apparently sensitizes this strain to many frameshifting mutagens. Only a few compounds appeared to exhibit microsomal activation and only one seemed to require such treatment. In addition to aglycones shown in Table 1, ten glycosides were also tested. The *O*-β-D-glucosides of three agents directly mutagenic for *Salmonella* TA1537 were non-mutagenic for any of the five tester strains. After treatment with crude liver microsomal preparations, some mutagenicity was seen for strain TA1537 with chrysazin monoglucoside, quinizarin monoglucoside and franguloside (emodin 6-*O*-rhamnoside). Alizarin 2-*O*-β-D-glucoside and emodin

Table 1. *Mutagenicity testing of some hydroxylated anthraquinones and related compounds in the 'Salmonella/microsome' test*

Mutagenicity is expressed in terms of revertants/nmol, i.e. the increase in numbers of revertants/plate per nmol increase in the quantity of test agent over the linear portion of the dose–response curve. Also given are typical mutagenic responses in terms of revertants/plate less background for a given quantity of test agent.

Test agent	Substitution	*His* revertants (no./nmol)*	*His* revertants (no./plate)†	Strain‡	Activation§
Anthrone	10-H_2	<0.01	—	—	−
9,10-Anthraquinone	—	<0.01	—	—	−
Anthralin	1,8-OH, 10-H_2	0.13	59 (100)	7	±
Anthrarobin	3,4-OH, 10-H_2	0.08	36 (100)	7	−
1-Hydroxyanthraquinone	1-OH	<0.01	—	—	−
	1-ONa, 5-SO_3Na	<0.01	—	—	−
Alizarin	1,2-OH	0.09	36 (100)	7	−
Alizarin Red S	1,2-OH, 3-SO_3Na	<0.01	—	—	−
Chrysarobin	1,8-OH, 3-CH_3, 10-H_2	0.10	40 (100)	7	±
Quinizarin	1,4-OH	0.32	134 (100)	7	−
	1,4-OH, 2-SO_3Na	0.08	150 (500)	7	−
Anthrarufin	1,5-OH	0.17	35 (50)	(7), 8, 9	−
Chrysazin	1,8-OH	0.41	172 (100)	7	−
Chrysophanol	1,8-OH, 3-CH_3	0.012	24 (500)	7	++
Anthraflavic acid	2,6-OH	<0.01	—	—	−
Diaminoanthrarufin	1,5-OH, 4,8-NH_2	1.8	520 (100)	7, 8, (9)	+
Dichloroanthrarufin	1,5-OH, 4,8-Cl	1.2	336 (100)	7, (8), 9	−
Dichloroquinizarin	1,4-OH, 5,8-Cl	<0.01	—	—	−
	1-OH, 2-O$[CH_2]_3SO_3Na$	0.06	90 (500)	(5), 0	−
Macrosporin	1,7-OH, 3-OCH_3, 6-CH_3	<0.01	—	—	−
	1,3-OH, 2-OCH_3	<0.01	—	—	−
Lucidin	1,3-OH, 2-CH_2OH	70	3600 (10)	(0), 7, 8, 9	−
Lucidin ethyl ether	1,3-OH, 2-$CH_2OC_2H_5$	82	2700 (10)	(0), 7, 8, 9	+
Deoxylucidin	1-OH, 2-CH_2OH	0.14	54 (100)	7, (8)	−
Aloe-emodin	1,8-OH, 3-CH_2OH	0.22	61 (100)	7	−
Leucoquinizarin	1,4-OH, 9,10-H	0.15	62 (100)	7	−
Dermoglancin	1,7,8-OH, 3-CH_3, 6-OCH_3	0.15	51 (100)	7	±
Solorinic acid	1,3,8-OH, 2-CO$[CH_2]_4CH_3$, 6-OCH_3	<0.01	—	—	−
Boletol	1,2(3),4-OH, 5-CO_2H	<0.01	—	—	−

Purpurin	1,2,4-OH	0.42	165 (100)	(7), 8, 9	−
Emodin	1,3,8-OH, 6-CH_3	1.8	330 (50)	7	+
Anthrapurpurine	1,2,7-OH	<0.01	—	—	−
Anthragallol	1,2,3-OH	2.0	800 (100)	7, 8, (9)	±
Quinalizarin	1,2,5,8-OH	0.53	97 (50)	7	±
Dermocybin	1,5,7,8-OH, 3-CH_3, 6-OCH_3	<0.01	—	—	−
	1,4-5,8-OH, 9,10-H	0.26	95 (100)	7	−
Rufigallol	1,2,3,5,6,7-OH	<0.01	—	—	−

* Revertants/nmol for most potent response observed for strain in parentheses; <0.01 revertant/nmol indicates a negative response.
† Revertants/plate less background for test agent (amount in μg in parentheses) tested in strain in parentheses.
‡ Strain code: 5, TA1535; 0, TA100; 7, TA1537; 8, TA1538; 9, TA98.
§ Activation by liver microsomal preparations from Aroclor 1254-induced rats: −, no activation; ±, weak or negative activation; +, significant activation; ++, activation obligatory for mutagenic activity.

Table 2. *Mutagenicity testing of some flavonoid compounds in the 'Salmonella/microsome' test*

See Table 1 for details.

		His revertants			
Test agent	Substitution	(no./nmol)	(no./plate)	Strain	Activation
Flavones					
Quercetin	3,3′,4′,5,7-OH	19.0	2400 (50)	(9), 0, 7	+
Myricetin	3,3′,4′,5,5′,7-OH	2.5	850 (100)	(9), 0, 7	+
Rhamnetin	3,3′,4′,5-OH, 7-OCH_3	0.45	159 (100)	(7), 9, 0	+
Kaempferide	3,5,7-OH, 4′-OCH_3	0.24	79 (100)	(7), 9	+
Fisetin	3,3′,4′,7-OH	0.12	42 (100)	7	+
Morin	2′,3,4′,5,7-OH	0.05	39 (250)	7	+
Robinetin	3,3′,4′,5′,7-OH	0.03	10 (100)	7	+
Apigenin	4′,5,7-OH	0.03	10 (100)	7	+
Diosmetin	3′,5,7-OH, 4′-OCH_3	<0.01	—	—	−
Galangin	3,5,7-OH	<0.01	—	—	−
Flavanones					
Taxifolin	3,3′,4′,5,7-OH	0.08	25 (100)	7	+
Eriodictyol	3′,4′,5,7-OH	<0.01	—	—	−
Hesperetin	3′,5,7-OH, 4′-OCH_3	<0.01	—	—	−
Naringenin	4′,5,7-OH	<0.01	—	—	−

1(8)-monoglucoside were non-mutagenic, as were the glucoside tetra-acetates of alizarin, chrysazin and quinizarin. When tested with the bacterial enzymes, all glucosides including the tetra-acetates were mutagenic for strain TA1537, apparently owing to formation of the free aglycones, alizarin, chrysazin, quinizarin and emodin, which are specific for strain TA11537. Emodin glucoside exhibited maximal activity after treatment with both bacterial and microsomal enzymes. Franguloside was not as clearly activated by the bacterial extracts as were the glucosides. This agent may act as some other metabolite of the parent glycone. In addition to the *O*-glycosides, two anthraquinone C-glucosides, namely aloin and carminic acid, were tested by the same procedures and found to be non-mutagenic.

Frameshift mutagenicity among the flavonoid compounds tested was mainly confined to the flavonols (flavon-3-ols) (Table 2). The flavonols are probably the single largest group of flavonoids, and the most mutagenic agent detected, quercetin, is the most common flavonol aglycone. Two quercetin glycones, rutin (quercetin 3-D-rutinoside) and quercitrin (quercetin 3-L-rhamnoside), were found to be very weakly mutagenic in strains TA100, TA1537 and TA98 with microsomal activation (as reported earlier by Hardigree & Epler, 1977). Mutagenic activity of the glycones for these strains could be increased 10–20-fold by incorporating gut bacterial enzymic extracts and microsomal enzymes in the assay procedure. It is still uncertain into what mutagenic intermediates the glycones are converted. Weak glycosidase activity in the S9 preparations could account for these results. A number of additional agents including four flavanone glycones (hesperidin, naringin, robinin and neoeriocitrin), 16 dihydrochalcones and three dihydrochalcone glycones (hesperetin dihydrochalcone glucoside, neohesperidin dihydrochalcone and neoeriocitrin dihydrochalcone), also were tested and found to be non-mutagenic.

The implication of possible genetic toxicity of hydroxy anthraquinones is not very surprising for this group of compounds in view of other forms of toxicity exhibited by many of its members (Kean, 1968; Uraguchi *et al.*, 1972; Wells *et al.*, 1975; Fairbairn, 1976). The same cannot be said for the flavonols, which, although having many physiological effects, are apparently non-toxic in man.

Ames, B. N., McCann, J. & Yamasaki, E. (1975) *Mutat. Res.* **31**, 347–364
Brown, J. P. & Brown, R. J. (1976) *Mutat. Res.* **40**, 203–224
Fairbairn, J. W., (ed.) (1976) *Pharmacology* **14**, *Suppl.* **1**, 1–101
Harborne, J. B., Mabry, T. J. & Mabry, H. (eds.) (1975) *The Flavonods*, pp. 377, 1011–1014, 1033–1036, Academic Press, New York
Hardigree, A. A. & Epler, J. L. (1977) *Mutat. Res.* in the press
Kean, E. A. (1968) *Arch. Biochem. Biophys.* **127**, 528–533
Mathis, C. (1966) in *Comparative Phytochemistry* (Swain, T., ed.), pp. 245–270, Academic Press, London and New York
Thomson, R. H. (1971) *Naturally Occurring Quinones*, 2nd edn., chapter 5, Academic Press, London and New York
Uraguchi, K., Saito, M., Noguchi, Y., Takahashi, K., Enomoto, M., & Tatsuno, T. (1972) *Food Cosmet. Toxicol.* **10**, 193–207
Wells, J. M., Cole, K. J. & Kirksey, J. W. (1975) *Appl. Microbiol.* **30**, 26–28

Desaturation of Fatty Acids by the Psychrophilic Bacterium *Micrococcus cryophilus*

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A wide range of organisms including prokaryotes and eukaryotes respond to growth temperature changes by altering their membrane lipid fatty acid composition. Such changes generally are believed to be part of a mechanism for retaining the fluid proper-