Note

Effects of Triterpenes on the Mutagenicities of Various Mutagens toward *Salmonella*Tsutomu YAMAGUCHI and
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With a view to securing our food supplies and environment, studies on the screening of factors suppressing mutagens have been made by many workers.¹⁻⁴⁾ We have described the antimutagenic action of horseradish peroxidase on autoxidized fatty acids.⁵⁾ Recently, we have also reported the reduction of induced mutagenicities of various mutagens with biologically active quinones⁶⁾ or xanthine derivatives.⁷⁾ Many of these substances were known to have an inhibitory activity on cyclic AMP phosphodiesterase [3.1.4.17]. From these results, we became interested in whether triterpenes, another inhibitor of this enzyme, would have the same effect or not. In the present paper, we determined the effects of various triterpenes on the mutagenicities of various mutagens.

A mutation assay was carried out according to the method reported by Ames *et al.*⁸⁾ with some modification.⁹⁾ Liver microsomal fractions were prepared from rats previously treated with PCB as described by Ames *et al.*¹⁰⁾ Cells of *Salmonella* were incubated at 37°C for 20 min with 0.1 ml of mutagen solution, with or without 0.25 ml of the S-9 mix and 0.1 ml of a triterpene sample solution, and the mutagenicity was tested by the standard method.⁹⁾

Benzo(a)pyrene (B(a)P), 2-acetyl aminofluorene (AAF), and 4-nitroquinoline-1-oxide (4NQO) were purchased from Sigma Chemicals Co. 3-Amino-1-methyl-5H-pyrido [2,3-b]indol (Trp-p-2) was kindly supplied by Dr. T. Kada, the National Institute of Genetics, Japan. All other reagents used were of guaranteed grade.

Table I(a) shows the effects of various triterpenes on the mutagenicities of Trp-p-2, AAF, and B(a)P, all of which show their mutagenicities by metabolic activation with S-9 mix.

The mutagenicities of these three greatly decreased when they were incubated with S-9 mix in the presence of one of these triterpenes. Glycyrrhizin was the most effective among these triterpenes. Reduction of survival by the

addition of these compounds was not significant in the range of concentrations tested.

Table I(b)-(1) shows the effects of these triterpenes on the mutagenicities of sugar-degraded compounds which show their mutagenicities without metabolic activation. Sharply in contrast with Table I(a), these triterpenes, except glycyrrhizin, strongly stimulated the mutagenicities of these sugar-degraded compounds. None of these tri-

FIG. 1. Dose-response Curves of Triterpenes on the Mutagenicities of (a) Trp-p-2 and (b) Methyl Glyoxal.

A reaction mixture contained (a) Trp-p-2 (0.1 µg/plate) with S-9 mix, test strain (*Salmonella* TA 98) and various concentrations of triterpenes, (b) Methyl glyoxal (20 µg/plate) without S-9 mix, test strain (*Salmonella* TA 100) and various concentrations of triterpenes. The numbers of revertants were: for Trp-p-2, 5123; methyl glyoxal, 1635. ●—●, glycyrrhizin; ○—○, glycyrrhetic acid; □—□, lanosterine; ▲—▲, squalene.

FIG. 2. Changes of the Reduction of Mutagenicities by the Concentrations of S-9 mix.

A reaction mixture contained a test mutagen, cells of the test strain, glycyrrhizin (200 µg/plate) and various concentrations of S-9 mix. The test strains used were *Salmonella* TA 98 for assay of Trp-p-2 and AAF, and TA 100 for that of B(a)P. ●—●, Trp-p-2; ○—○, AAF; □—□, B(a)P.

TABLE I. EFFECTS OF VARIOUS TRITERPENES ON THE MUTAGENICITIES OF VARIOUS MUTAGENS

The concentrations of all these samples were 200 µg/plate. The test strains were *Salmonella typhimurium* TA 98 for the assay of Trp-p-2 and AAF, and TA 100 for the assay of all the other mutagens. The concentrations of the mutagens/plate were for Trp-p-2, 0.1 µg; AAF, 10 µg; B(a)P, 5 µg; methyl glyoxal, 20 µg; glyceraldehyde, 100 µg; glucose pyrolysate, 0.1 ml of 150-fold diluted solution; AF-2, 0.06 µg; 4NQO, 5 µg; glycidol, 500 µg; propylene oxide, 350 µg.

(a) Mutagens which show their mutagenicities by metabolic activation with S-9 mix

	Revertants/plate			Surviving cells ($\times 10^7/0.1$ ml)
	Trp-p-2	AAF	B(a)P	
Control	4,955	3,754	386	1.83
Glycyrrhizin	96	388	25	1.85
Glycyrrhetinic acid	143	370	33	1.87
Squalene	717	634	58	1.82
Lanosterine	1,101	740	91	1.83

(b) Mutagens which show their mutagenicities without metabolic activation

(1) Sugar-degraded compounds

	Revertants/plate			
	Methyl glyoxal	Glyceraldehyde	Glucose pyrolysate ^a	None
Control	1,620	658	572	—
Glycyrrhizin	719	520	147	23
Glycyrrhetinic acid	3,213	1,625	755	78
Squalene	5,095	3,445	1,395	28
Lanosterine	5,278	4,049	2,025	33

(2) Other compounds

	Revertants/plate			
	AF-2	4NQO	Glycidol	Propylene oxide
Control	938	2,234	1,232	536
Glycyrrhizin	967	2,083	1,253	615
Glycyrrhetinic acid	923	2,125	1,125	562
Squalene	895	2,216	1,093	495
Lanosterine	972	2,253	1,223	528

^a See ref. 11.

terpenes showed mutagenicity on *Salmonella typhimurium* TA 98 and TA 100.

Table I(b)-(2) shows the effects of triterpenes on the mutagenicities of the compounds, besides sugar-degraded compounds, which also show their mutagenicities without metabolic activation. Triterpenes neither decreased nor stimulated the mutagenicities of the mutagens in this group. It is a phenomena of great interest that triterpenes cause the reduction of mutagenicities on some mutagens, they stimulate some mutagens, and they have no effect on some mutagens. Glycyrrhizin, that is the glycoside of

glycyrrhetinic acid, was exceptional among the triterpenes tested in that it did not stimulate the mutagenicities of sugar-degraded compounds but rather decreased them.

Figure 1(a) shows the dose-response curves of various triterpenes on the mutagenicity of Trp-p-2.

With all of these triterpenes, the mutagenicity was markedly decreased in accordance with the concentrations, and was completely lost above about 200 µg/plate.

Figure 1(b) shows the dose-response curves of various triterpenes on the mutagenicity of methyl glyoxal. The

mutagenicity was stimulated by the presence of most of these triterpenes with increasing concentrations.

Figure 2 shows the effects of glycyrrhizin on the reduction of the mutagenicities of Trp-p-2, AAF, and B(a)P when they were activated by various concentrations of S-9 mix.

The optimal dose of S-9 mix for revelation of the mutagenicities of these mutagens was 10 to 15 μ g/plate (data not shown). For the reduction of their mutagenicities, glycyrrhizin was the most effective at a lower dose of S-9 mix than the optimum for the revelation. When higher levels of S-9 mix were added, less effect of glycyrrhizin was observed. Apart from these mutagens, which require S-9 for their activation, the direct mutagen prepared from Trp-p-2 by treatment with S-9³⁾ was not inhibited by these triterpenes. It would be probable that the effect of triterpenes may be due to their interference with the S-9 metabolic activation.

Further studies might clarify how these triterpenes act against these mutagenicities.

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