Short Communication

Bio-antimutagenic Effect of Lactones
on Chemical Mutagenesis in
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Received June 12, 1985

Some plants contain substances that suppress the process of mutagenesis.^{1,2)} Kada *et al.* called these compounds bio-antimutagens and found that lactones such as coumarin, umbelliferone, and protoanemonin have bio-antimutagenic effects using a microbial bioas-

say system.^{3,4)} Here we describe the bio-antimutagenic effect of twenty-three lactones.

Chemicals were obtained from the following sources: γ -butyrolactone, γ -valerolactone, α -angelica lactone, α -acetyl butyrolactone, α -methylene butyrolactone, γ -decalactone, δ -valerolactone, 5,6-dihydro-2*H*-pyran-2-one, phthalide, 2-coumaranone, δ -glucuronolactone, esculetin, 4-methylesculetin, esculin, 6-methylcoumarin, 7-methylcoumarin, dihydrocoumarin, 3-acetylcoumarin, 5,7-dimethoxycoumarin, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) from the Aldrich Chem. Co.; γ -hydroxy-*n*-caprylic acid lactone and 4-nitroquinoline 1-oxide (4NQO) were from the Tokyo Kasei Kogyo Co. β -Angelica lactone, pantolactone, and cyclohexyllactone were generous gifts from Dr. Shigematsu in our laboratory.

Escherichia coli B/r WP2 (*trp*⁻) and WP2s (*trp*⁻, *uvrA*⁻) were used as the test strains. Lactones were bioassayed quantitatively

TABLE I. EFFECT OF LACTONES ON MUTAGENESIS CAUSED BY 4-NITROQUINOLINE-1-OXIDE (4NQO) IN WP2 OR WP2s, AND BY *N*-METHYL-*N*'-NITRO-*N*-NITROSOGUANIDINE (MNNG) IN WP2

No. Name	Dose (mg/plate)	4NQO (30 μ g/ml)		4NQO (2 μ g/ml)		MNNG (8 μ g/ml)	
		Revertants of WP2/ plate	Viable cells (%)	Revertants of WP2s/ plate	Viable cells (%)	Revertants of WP2/ plate	Viable cells (%)
None		332	100	4042	100	1998	100
1 γ -Butyrolactone	1	343	102	4328	104	2076	98
	2	313	101	4253	93	1956	99
	3	296	97	4199	95	2001	95
2 γ -Valerolactone	1	296	103	4102	101	1856	102
	2	325	108	4373	90	1996	105
	3	296	106	4373	101	2003	96
3 α -Acetyl butyrolactone	0.5	296	101	4282	95	1967	106
	1	329	92	4396	87	1973	91
	2	323	85	4120	84	1752	84
4 α -Methylene butyrolactone	1	98	99	574	99	620	103
	2	31	110	105	97	134	99
	3	13	101	17	98	15	101
5 γ -Decalactone	0.5	365	106	4008	93	2032	105
	1	328	105	3848	95	1998	96
	2	250	82	3368	80	1902	81
6 γ -Hydroxy- <i>n</i> - caprylic acid lactone	1	330	109	3976	102	1908	98
	2	314	108	3854	96	1767	99
	3	340	108	4054	91	2001	105
7 α -Angelica lactone	1	322	107	4080	96	1890	96
	2	317	96	3767	93	1768	101
	3	314	103	2897	102	1656	98

TABLE I. (continued)

No. Name	Dose (mg/plate)	4NQO (30 μ g/ml)		4NQO (2 μ g/ml)		MNNG (8 μ g/ml)	
		Revertants of WP2/ plate	Viable cells (%)	Revertants of WP2s/ plate	Viable cells (%)	Revertants of WP2/ plate	Viable cells (%)
8 β -Angelica lactone	1	333	104	4332	101	1897	101
	2	334	106	4118	103	1899	99
	3	321	109	2959	104	1806	105
9 Pantolactone	1	335	106	4225	105	1978	98
	2	320	104	4119	100	1970	99
	3	320	103	4001	99	1999	103
10 δ -Valerolactone	1	303	105	3776	93	1935	102
	2	296	106	2998	101	1950	103
	3	295	104	3116	98	1820	99
11 5,6-Dihydro-2H- pyran-2-one	1	130	100	1823	98	1011	101
	2	79	98	988	102	924	103
	3	56	108	331	99	424	98
12 Phthalide	1	335	97	4153	99	1923	105
	2	331	106	4172	98	1872	97
	3	328	103	4024	104	1608	102
13 2-Coumaranone	1	318	103	3728	103	1923	101
	2	303	104	3784	98	1999	105
	3	305	98	3370	101	1976	98
14 δ -Glucuronolactone	1	336	96	4213	98	1976	101
	2	301	102	4115	101	1988	104
	3	284	98	3978	106	2056	106
15 Esculetin	1	175	107	1992	102	1482	102
	2	122	97	980	103	970	101
	3	80	101	652	108	334	103
16 4-Methylesculetin	1	294	105	1652	93	1566	97
	2	108	103	484	101	936	101
	3	52	96	277	107	352	98
17 Esculin	1	332	95	4353	102	1943	107
	2	298	101	4437	106	1935	103
	3	304	99	4445	95	1894	101
18 6-Methylcoumarin	1	338	96	2944	97	1923	101
	2	341	99	2728	100	1876	103
	3	334	104	2106	99	1044	105
19 7-Methylcoumarin	1	335	95	3888	98	1911	99
	2	331	106	4072	105	1923	101
	3	335	99	2201	101	1311	99
20 Dihydrocoumarin	1	298	104	3675	104	1978	98
	2	311	97	3392	98	1968	98
	3	298	103	2898	102	1953	105
21 Cyclohexyllactone	1	341	106	4253	101	1935	99
	2	328	107	4414	104	1958	98
	3	304	102	4352	99	1976	103
22 3-Acetylcoumarin	1	336	98	3904	98	1995	101
	2	320	104	2634	99	1895	98
	3	336	99	2543	104	1780	107
23 5,7-Dimethoxy- coumarin	0.5	324	101	4117	98	1968	98
	1	299	101	4233	96	1996	95
	2	335	96	4397	103	1950	106

Data points of the test substances are the means from 3 experiments.

TABLE II. SENSITIVITIES OF INDUCED Trp⁺ COLONIES OF *E. coli* B/r WP2 *trp*⁻ TO LACTONES UNDER RECONSTRUCTED CONDITIONS

Name	Concentration (mg/plate)	Mean number of Trp ⁺ colonies/plate ^a	Survival (%)
None	0	1100	100
α -Methylene butyrolactone	1	1026	102
	2	1075	101
	3	1092	100
4-Methyl-esculetin	1	1052	102
	2	1106	103
	3	1093	101
Esculetin	1	1089	100
	2	1120	99
	3	1099	103
5,6-Dihydro-2H-pyran-2-one	1	1098	100
	2	1102	102
	3	1105	101

^a Individual points are the means from counts of revertants on 2 plates.

by methods described elsewhere.^{5,6)} Bacteria grown overnight in nutrient broth at 37°C were washed twice with phosphate buffer and resuspended in phosphate buffer. The cells were treated with 4NQO or MNNG at 37°C for 30 min. The treated cells were washed twice with phosphate buffer and resuspended in phosphate buffer ($3 \sim 4 \times 10^9$ cells/ml). A 0.1 ml portion of the treated cell suspension and various amounts of lactones were mixed well in 2 ml of molten top agar, and the mixture was poured onto a semi-enriched minimal agar plate. To measure cell viability, the suspension of the treated cells was diluted by 10^6 and 0.1 ml of the diluted suspension was poured onto a plate as described above. Trp⁺ revertants and viable cells were counted after incubation at 37°C for 2 days.

The results of the screening are summarized in Table I. The lactones were not mutagenic with WP2 and WP2s (data not shown). Of the lactones tested, α -methylene butyrolactone,

5,6-dihydro-2H-pyran-2-one, esculetin, and 4-methylesculetin had bio-antimutagenic effects on 4NQO- or MNNG-induced mutagenesis in WP2 and on 4NQO-induced mutagenesis in WP2s. The number of Trp⁺ revertant colonies decreased as the concentration of lactones increased. The lactones were not toxic to the cells within the concentration tested. To examine inhibition by α -methylene butyrolactone, 5,6-dihydro-2H-pyran-2-one, esculetin, and 4-methylesculetin of the expression of Trp⁺, a reconstruction experiment was done as described elsewhere.⁶⁾ There was no inhibition of this phenotype (Table II). The Trp⁺ colonies grew well even in the presence of these substances. Therefore, the bio-antimutagenic effects of these compounds are not due to selective killing of Trp⁺ revertants.

The bio-antimutagenic effect of α -methylene butyrolactone was the strongest of the lactones tested. The $\text{CH}_2=\text{C}-\text{CO}-$ group reacts with enzyme-SH groups.⁷⁾ The strength of this effect of α -methylene butyrolactone may be attributable to the reactivity of this group, which it contains.

Acknowledgment. We are grateful to Dr. T. Kada for providing us with the bacterial strains.

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