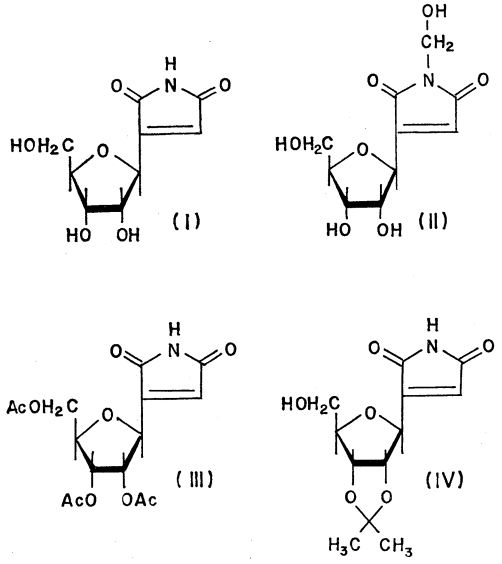
Communication to the Editor

EFFECTS OF CHEMICAL MODIFICATION ON RADIOSENSITIZATION OF *E. COLI* B/r BY SHOWDOMYCIN

Sir:

Showdomycin (I) has a marked radiosensitizing effect on *Escherichia coli* B/r at 0.1 mM, the dose-modifying factor (D.M.F.) at this concentration in aerated condition being higher than that of N-ethylmaleimide[1]. Recently, the same type of equimolecular reaction which thiol compounds undergo with N-ethylmaleimide was seen to occur with showdomycin[2]. To know further about the properties of showdomycin in irradiated living systems, we have investigated the radiosensitizing effects of showdomycin and three kinds of derivatives, *viz.* showdomycin triacetate (**III**), showdomycin acetonide (**IV**), and N-methylolshowdomycin (**II**), in aerated and in anoxic conditions with ^{60}Co γ-rays using *E. coli* B/r as a model organism.

N-Methylolshowdomycin was prepared by methylolation of showdomycin at pH 5 according to the method essentially the same as that of TAWNEY *et at.*[3], being obtained as a hygroscopic, extremely water-soluble solid (Found: C 46.11, H 4.95, N 5.29. Calcd.: C 46.33, H 5.02, N 5.41). Its mass spectrum exhibited no molecular ion peak but exhibited an M-H_2O peak at m/e 241[4]. The acetonide and the triacetate of showdomycin were prepared according to the method of NAKAGAWA *et al.*[5]



Anoxic conditions were obtained by bubbling 99.999 % nitrogen gas into the cell suspension, with or without test compound, for 5~10 minutes at ice-cold temperature. Longer bubbling time did not give more complete anoxia in our system. The cell suspensions were then transferred into irradiation vessels under the nitrogen gas, being then kept tightly sealed until the end of irradiation. The irradiation and subsequent procedures have been described elsewhere[1].

First of all, the dose-survival curves of *E. coli* B/r in aerated and in anoxic conditions were determined in the presence and absence of showdomycin, and all of these curves were obtained as straight lines by our experimental condition, as shown in Fig. 1. The radiation dose under which there was 10 % survival (D_{10}) was then measured on each curve to calculate D. M. F. Assuming that these dose-survival curves would also be obtained as straight lines for other compounds, the surviving fraction at 40 krads were measured for three showdo-

Fig. 1. Dose-survival curves of *E. coli* B/r irradiated with ^{60}Co γ-rays in the presence and absence of showdomycin in aerated and in anoxic condition.

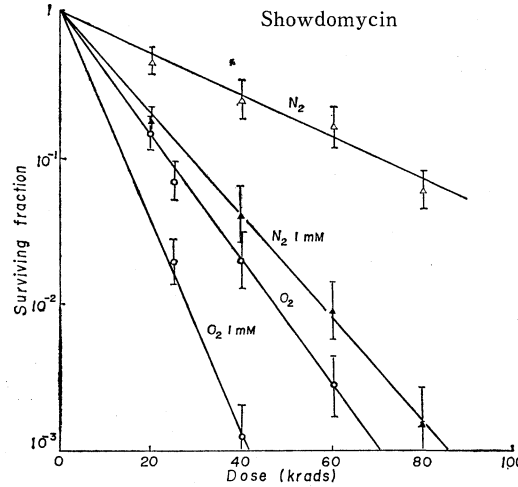


Table 1. Radiosensitization of *E. coli* B/r by showdomycin and its derivatives in aerated and in anoxic conditions

Test compound (1 mM)	M. I. C.* (μg/ml)	Aerated		Anoxic	
		D_{10} (krads)	DMF**	D_{10} (krads)	DMF
None		29 ± 5		81 ± 10	
Showdomycin (SHM)	1.5	18 ± 5	1.6±0.05	32 ± 5	2.5±0.2
SHM-triacetate	150	28 ± 5	1.0±0.05	32 ± 6	2.5±0.2
SHM-acetonide	15	27 ± 5	1.1±0.05	31 ± 1	2.6±0.2
N-methylol-SHM	3	23 ± 5	1.3±0.05	25 ± 1	3.2±0.2

* Minimal inhibitory concentration (M. I. C.) was determined for *E. coli* B/r by tube dilution method after 24 hours at 37℃ in synthetic medium (NH_4Cl 1 g, NaCl 5 g, Na_2HPO_4 6 g, KH_2PO_4 3 g, $MgSO_4$ 0.1 g, Glucose 10 g in 1,000 ml distilled water, pH 7.0).

** $DMF = \frac{D_{10} \text{ in the absence of test compound}}{D_{10} \text{ in the presence of test compound}}$

mycin derivatives with one buffer control in aerated and in anoxic condition; then D_{10} values were determined using these points, and D.M.F.s were calculated according to an equation demonstrated in Table 1. The mean values of three independent experiments are summarized in Table 1. As shown in the table, D_{10} values deviated about ± 20 % in some cases, however, D.M.F.s were obtained in very good accuracy in all cases, because D_{10} values for both control and test compounds deviated in the same direction in each experiment.

As was expected from our previous report[1], the D.M.F. of showdomycin was increased by deoxygenation of the system. Although the radiosensitizing effect of showdomycin in aerated system was reduced by its chemical modification, in anoxic systems modification of the sugar moiety did not reduce the sensitizing effect, and N-methylolation of the maleimide moiety enhanced the effect. These facts suggest that the radiosensitizing ability of showdomycin and its derivatives in anoxic condition is mainly derived from the maleimide moiety, and the mode of action is similar to that of the N-ethylmaleimide[6], being independent of the sugar side chain. In contrast, in aerated condition, hydroxy groups of sugar moiety, which are probably essential to the antibiotic activity of showdomycin, seem to be also essential for the appearance of the radiosensitizing ability.

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