

## EQUIMOLECULAR REACTION OF SHOWDOMYCIN WITH THIOLS\*

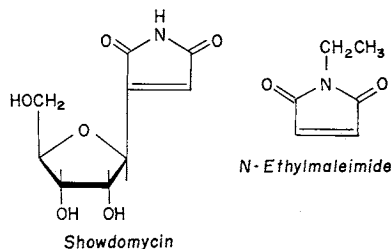
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Showdomycin, first isolated from *Streptomyces showdoensis* in our laboratory<sup>1)</sup>, inhibits the growth not only of Gram-positive and Gram-negative bacteria but also of mammalian cells<sup>1-2)</sup>; its structure was found to be 3 $\beta$ -D-ribofuranosyl maleimide, as shown in Fig. 1<sup>3-4)</sup>. Unsaturated imides such as N-ethylmaleimide react rapidly with thiols<sup>5)</sup>, and this report shows that showdomycin also reacts stoichiometrically with sulfhydryl compounds in aqueous solution. The determination of SH groups was carried

Fig. 1. Structure of showdomycin and N-ethylmaleimide.



out according to ELLMAN's method<sup>6)</sup>. When varying concentrations of showdomycin reacted with excess of thiols, the molar amounts of thiol and antibiotic consumed were equal, as shown in Table 1. Similar results were obtained in the reaction of N-ethylmaleimide and thiols. The addition product of N-ethylmaleimide and cysteine was first isolated by SMYTH *et al.*<sup>7)</sup> as S-(N-ethyl-succinimido)-L-cysteine. Following their method, we isolated the product of showdomycin and cysteine. NMR data and elemental analysis of the product suggested

Table 1. Equimolecular reaction of showdomycin with thiols.

	(A) Thiols (mM)	(B) SH reagents added (mM)	(C) SH groups remaining (mM)	SH groups consumed (A - C) (mM)	Ratio of SH groups consumed to SH reagent added $\left(\frac{A-C}{B}\right)$
Exp. 1	Cysteine 0.164	NEM 0.041	0.122	0.042	1.0
		0.082	0.076	0.088	1.1
		0.123	0.044	0.120	0.98
	SHM	0.041	0.125	0.039	0.95
		0.082	0.085	0.089	1.1
		0.123	0.042	0.122	0.99
	GSH 0.246	SHM 0.050	0.198	0.048	1.0
		0.100	0.149	0.097	1.0
		0.150	0.105	0.145	1.0
0.200		0.055	0.195	1.0	
Exp. 2	GSH 0.224	SHM 0.050	0.175	0.049	0.98
		0.100	0.136	0.088	0.88
		0.150	0.086	0.138	0.92
		0.200	0.041	0.183	0.92

The reactions between SH reagents and thiols were carried out in water (Exp. 1) or 0.05 M Tris-HCl (pH 7.5) (Exp. 2) at room temperature. Sulfhydryl determinations were carried out with 5,5'-Dithiobis (2-nitrobenzoic acid) in phosphate buffer at pH 8.0 according to ELLMAN's method.<sup>6)</sup> The sulfhydryl content was calculated assuming an  $E_{m}$  of 13,600 at 412 m $\mu$  for the reaction product.

Abbreviations used: NEM, N-ethylmaleimide; SHM, showdomycin; GSH, glutathione.

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that showdomycin reacts with an equimolecular proportion of thiol by the addition of SH group to the double bond in maleimide to give S-(3 $\beta$ -D-ribofuranosyl succinimido)-L-cysteine. These results thus show that showdomycin reacts stoichiometrically with an equimolecular ratio of thiol.

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