

INHIBITION OF FOCUS FORMATION OF
RAT CELLS BY MOUSE SARCOMA
VIRUS BY DAMAVARICIN
Fc DERIVATIVES

Sir:

Streptovaricins were discovered by SIMINOFF *et al.* and have been studied for their potential antitubercular activity¹. A chemical study of streptovaricins was made by RINEHART and his collaborators and the relationship between chemical structure and biological activity was explored². In addition to antibacterial activity, inhibition of reverse transcriptase of RNA tumour virus was observed in streptovaricins³. There is evidence that streptovaricins block the transformation of mouse cells by MOLONEY murine sarcoma-leukemia complex *in vitro*⁴.

We have been interested in the various kinds of damavaricin C (DvC) derivatives which have been derived from streptovaricin C (SvC) as reported previously⁵. DvC has a newly formed phenolic hydroxyl group at the C-19 position of the naphthoquinone ring in the molecule, thus enabling us to prepare various derivatives having an alkyl ether linkage at this position. Thus obtained DvC derivatives retain their antibacterial activity, but acquire cell killing activity for mammalian cells, including viral transformed cells and human leukemia cells, *in vitro*⁶. Because of their cell killing activity, DvC derivatives have not yet been examined for an intriguing property, that is the inhibition of focus formation by RNA tumour virus.

During the course of the preparation of DvC by treatment of SvC with oxygenated concentrated ammonia - methanol (1:2), at the same time was produced "damavaricin Fc" (DvFc, **1**) which

was found to be an atropisomeric mixture of two compounds, damavaricin Fc (**1a**) and atropisodamavaricin Fc (**1b**)⁷, at a ratio of 1:3 [$C_{36}H_{43}NO_{12}$, mp 224~226°C, $[\alpha]_D^{30} -532^\circ$ (c 0.11, $CHCl_3$)], the fact having been suggested from the peak intensities of the two phenolic hydroxyl protons in the pmr spectrum (unpublished data). Damavaricin Fc (**1a**), in which the ansa bridge lies above the aromatic nucleus, has the P helicity as well as natural streptovaricins, whereas atropisodamavaricin Fc (**1b**), in which the ansa bridge lies below the aromatic nucleus, has the M helicity as shown in Fig. 1. To avoid complicating nomenclature, hereafter we will use DvFc to refer to the atropisomeric mixture of damavaricin Fc. DvFc (**1**) has a δ -lactone ring between C-7 and C-10 of the ansa bridge which has been proved to produce a change in one of the biological properties. Specifically, the loss of antibacterial activity implies that DvFc has lost its capacity to bind RNA polymerase of prokaryotic cells. The inhibitory activity of the reverse transcriptase, however, is retained. These observations have prompted us to prepare various derivatives of DvFc as well as DvC and to examine their biological activity. DvFc has also an additional phenolic hydroxyl group at the C-19 position of the naphthoquinone ring, enabling the derivatization at this position as shown in Fig. 1.

It is of interest that DvFc derivatives are less toxic against animal cells *in vitro* as compared with DvC derivatives. Therefore we were able to test whether or not any of the derivatives inhibited focus formation by MSV/MLV complex. As shown in Table 1, methyl (**2**), ethyl (**3**), and *n*-pentyl (**4**) ethers of DvFc were effective, whereas benzyl ether (**7**) was not effective.

Fig. 1.

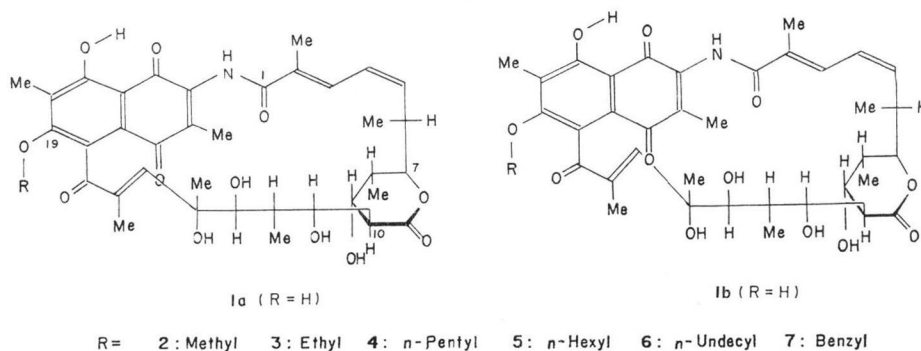


Table 1. Inhibition of focus formation by MSV/MLV and inhibition of RDDP.

DvFc derivative	% Inhibition of focus formation at 5 μ g/ml		% Inhibition of RDDP at 30 μ g/ml
	focus in the presence of drug	focus in the absence of drug	
2	76/111	28.2%	56
3	39/59	20 %	34
4	64/111	42.3%	60
7	48/51	6.0%	0

NRK cells were grown to 1.5×10^5 cells/dish in 20 cm² Falcon Petri dishes in EAGLE'S MEM supplemented with 10% fetal calf serum and 10% tryptose phosphate broth.

Cells were infected with MOLONEY'S mouse sarcoma virus and Polybrene was added to a final concentration of 2 μ g/ml. At the same time the derivatives were added at a concentration of 5 μ g/ml. After three days' incubation, the medium was removed and the fresh medium was added. After further three days' incubation, the cells were fixed and stained. The activity of reverse transcriptase was measured as follows. The reaction mixture in a final volume of 0.1 ml containing: 0.03 M Tris-HCl buffer (pH 8.1); 0.024 M KCl; 0.01 M MnCl₂; 0.5×10^{-3} M dATP, dCTP, dGTP; 0.025% NP40; 5 mM dithiothreitol; 7.5 mM [³H]-dTTP (New England Nuclear Corp., 1 Ci/mol). Samples were incubated at 37°C for 60 minutes and the reaction was terminated by addition of 1 ml of cold 10% trichloroacetic acid (TCA). The precipitate were then washed extensively with cold 5% TCA on "Millipore" filters, dried and counted in a liquid scintillation counter. DvFc derivatives were dissolved in methanol at a concentration of 6 mg/ml.

Table 2. Inhibition of the growth of mouse leukemia virus.

Exp.	Drug	Concentration (μ g/ml)	Cell No./dish ($\times 10^6$)	Relative activity	
				c.p.m.	%
I	no drug	—	2.9	2972	100
	4	5	1.6	87	2.9
II	no drug	—	4.8	3920	100
	4	3	4.3	180	4.6
III	no drug	—	4.4	3784	100
	1	4	4.2	3917	104
	2	5	4.9	2773	73
	5	4	4.5	2689	71
	6	5	4.4	3784	100

NRK cells were grown to 1.5×10^5 cells/dish as described in the legend to Table 1. Cells were infected with MOLONEY mouse leukemia virus. After four days' incubation, the culture fluid was collected by centrifugation at 25,000 r.p.m. overnight. The white band which formed at the density of 1.159 was collected and resuspended in TEN buffer. The RDDP activity of the obtained viruses was measured as described in the legend to Table 1.

It is noteworthy that the inhibitory activity of the derivatives for the reverse transcriptase (RDDP) *in vitro* was related to their inhibitory activity on focus formation by mouse sarcoma and mouse leukemia virus complex.

Next, the effect of the derivatives on the growth of mouse leukemia virus was examined. Normal rat cells were infected by mouse leukemia

virus. After four days, the virions were collected from the culture fluid by centrifugation and the growth of leukemia virus was compared by measuring the activity of the reverse transcriptase in the virions. As shown in Table 2, the derivatives used in this experiment were not toxic against cell growth, but the growth of the virus-infected cells was slightly inhibited. Al-

though methyl (2), *n*-hexyl (5), *n*-undecyl (6) ethers and DvFc itself did not inhibit viral growth, *n*-pentyl ether (4) did. The hydrophobic groups introduced into C-19 position of DvFc seem to confer the properties of penetrability and selectivity for the cells.

It has been reported that the inhibitory activity of the streptovaricins for reverse transcriptase *in vitro* is weak compared with rifamycin^{8,9,10}. Results of *in vivo* experiments using streptovaricins in the past, however, were very interesting. It was reported that the growth of mouse mammary tumours was significantly inhibited by the oral administration of streptovaricin complex¹¹ and that the oral administration of streptovaricin complex reduced RAUSCHER leukemia virus induced splenomegaly¹².

In this context, we believe it is worthwhile to study the DvFc derivatives *in vivo*. Fortunately the acute toxicity of the DvFc derivatives is remarkably reduced compared with streptovaricin or damavaricin C derivatives (unpublished data). Experiments along this line are in progress now.

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