Acta virol. **24**: 445–450, 1980

THE EFFECT OF TRIAZOLE ON RNA VIRUSES

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Received January 8, 1980; revised May 11, 1980

Summary. — The triazole derivative 4-(1-hydroxy-3-piperid-N-yl-prop-1-yl)-5-methyl-2-phenyl-triazole (HMPT) markedly reduced plaque formation by, RNA bacteriophages and delayed their liberation. Replication of tobacco mosaic virus in a systemic host was hardly affected by HMPT, but local lesion formation in a hypersensitive host was strongly reduced by the drug. The mechanism of action of HMPT was discussed.

Key words: triazole derivative; antiviral effects; RNA phages; tobacco mosaic virus

Introduction

Of the few triazole derivatives with an antiviral activity found so far, Virazole (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) shows the broadest and strongest inhibitory activity against zoo- and phytopathogenic viruses (Sidwell *et al.*, 1972; Witkowski *et al.*, 1972; Khurana *et al.*, 1975; Schuster, 1976; Lerch, 1977; Kluge and Marcinka, 1979; etc.). But bacterial viruses were hardly affected by Virazole (Menzel and Stenz, 1978). These different effects of Virazole on viruses of eukaryotes and prokaryotes prompted us to test the triazole derivative 4-(1-hydroxy-3-piperid-N-yl-prop-1-yl)-5-methyl-2-phenyl-triazole, the antiviral activity of which was demonstrated in screening experiments (Menzel and Kluge, 1979), in different virus-host systems. For sake of comparison, only RNA viruses were used.

Materials and Methods

Substances. 4-(1-Hydroxy-3-piperid-N-yl-prop-1-yl)-5-methyl-2-phenyl-triazole (HMPT), synthesised by Prof. B. Hirsch and Dr. D. Lohmann, Technical University, Dresden, and its component piperidine were tested, with the exception of diffusion tests, in separate experiments. As shown by repeated experiments, the results were not substantially affected by the ifferent starting virus concentrations used.

Viruses. RNA phages M12, f2 and Qβ and green strain of tobacco mosaic virus (TMV) were used. For their origin see Kluge *et al.* (1978). The phages were grown in *Escherichia coli* strains W 1665 F+, K 1046 and AB 301. TMV was tested in *Nicotiana tabacum* cv. Samsun (systemic host) and *N. glutinosa* (local lesion host).

Bacteriophage assay. The effects on phage replication were investigated first in agar diffusion tests. Holes punched into agar double layers were filled with a 1% aqueous solution of the test substance and the overlay was inoculated with a suspension of an *E. coli* strain with a diluted

Table 1. Inhibition of plaque formation and multiplication of E. coli by HMPT and piperidine in diffusion test

Substance	Diameter of the inhibition zone (mm)					
	Phage			E. coli		
	M12	f2	Qβ	W 1665 F+	K 1046	AB 301
HMPT	5.5	5.5	5.0	2.0	1.5	2.0
Piperidine	3.0	2.0	2.0	0	0	0

phage lysate. Within the concentration gradient formed the zones of bacteria and phage growth inhibition could be determined (for details see Menzel *et al.*, 1975). In addition, we used the pour plate test for quantitative assay of the effect of HMPT on plaque formation. Without inoculation of phages into the overlay, this test offered information about the effects of known substance concentrations on bacterial growth. The effects of the substances were also investigated on free phages, on phage adsorption and in one-step growth eperiments (for methods see Menzel and Stenz; 1979).

TMV assay. The test plants were grown under constant conditions (Kluge, 1976). When investigating the effects of the substances on TMV replication, *N. tabacum* cv. Samsun plants were sprayed 2 days before and 2 days after inoculation three times each with aqueous solutions of HMPT (1 %) or piperidine (0.25 %). The virus contents were determined serologically (drop precipitin test) and by the local lesion assay, in the inoculated leaves 6 days and in secondarily infected leaves 13 days after inoculation (p.i.) (for details see Kluge *et al.*, 1978). To test the effects of the substances on local lesion formation, two leaves were detached from each *N. glutinosa* plant. On to their left halves, either a 1 % HMPT or a 0.25 % piperidine solution was applied with a brush; the right halves were treated similarly with water. Ten minutes after treatment, both halves were inoculated with TMV (sap diluted 1:1000). The local lesions were counted 3 days p.i. The effect of the substances on free TMV virions was tested by diluting purified TMV (titre of 1024) 1:1000 with 0.067 M phosphate buffer, pH 7.2, containing HMPT or piperidine, to reach final concentrations of 0.1 % HMPT or 0.025 % piperidine. After incubation for 10 min, the mixtures were subjected to local lesion assay.

Results

HMPT markedly affected plaque formation by M12, f2 and Qβ phages. In the diffusion test, the inhibition of plaque formation was similar with all three pages (Table 1). The inhibition of the growth of the bacterial hosts was less marked. Piperidine also reduced plaque formation, but had no effect on the growth of *E. coli*.

Table 2. Effects of HMPT and piperidine on the plaque numbers of f2 phage in E. coli AB 301 (plate test)

Concentration %	Plaque numbers (in % of control)	
	HMPT	Piperidine
0.10	1.5±0.2	54.9±2.3
0.09	2.6±0.4	
0.08	7.2±0.6	
0.07	11·1±1.2	
0.06	26.7±1.5	
0.05	39.7±2.6	73.5±2.6
0.4	79.5±1.5	
0.03	84.1±4.9	
0.02	86.6±3.2	
0.01	102.6±4.3	100.1±3.3

Table 3. Effects of HMPT and piperidine on the adsorption of f2 phage on to E. coli AB 301

Substance	Concentration %	Phages/ml × 10^{-8} in adsorption mixture	Non-adsorbed phages per ml × 10^{-6}	Calculated amount of phages adsorbed (%)
HMPT	0	2.3	5.1±0.7	97.8
	0.1		12.4±1.4	94.6
Piperidine	0	3.3	9.8±0.7	97.1
	0.1		16.2±1.8	95.1

The reduction of plaque formation in relation to HMPT and piperidine concentration is shown in Table 2. HMPT in a 0.1 % (2.95 mM) concentration reduced the plaque number to 1.5 %, but piperidine in a 0.1 % (11.7 mM) concentration. i.e. in a 4-fold higher molar concentration, reduced it only to 54.9 %. The multiplication of bacteria was not visibly affected by this concentration.

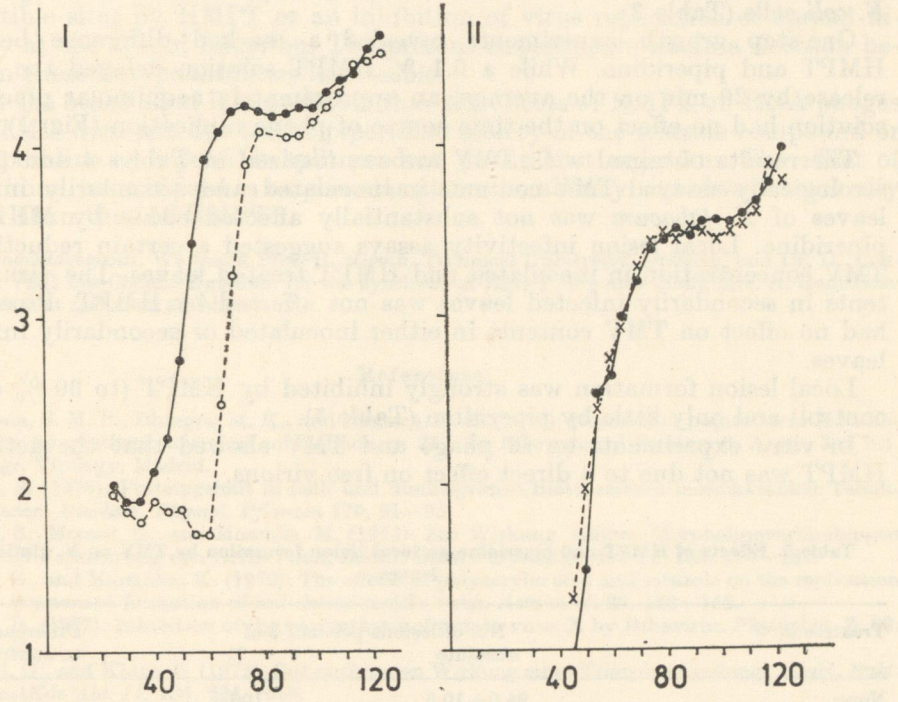

Fig. 1.

Effects of HMPT (0.1 %; ○) and piperidine (0.025 %; ×) on f2 phage in *E. coli* AB 301 (one-step growth experiments)

● — Controls

Abscissa: time in min; ordinate: log PFU/ml

Table 4. Effects of HMPT and piperidine on TMV replication in N. tabacum cv. Samsun

Treatment	TMV titre* I	TMV titre* II	No. of local lesions per half leas I	Difference to control**	No. of local lesions per half leas II	Difference to control**
None	6.3	6.3	22.2±4.0		28.1±4.0	
HMPT	5.8	6.2	13.2±6.4	−9.0	26.8±4.3	−1.3
None	5.3	7.8	7.6±2.0		15.0±5.2	
Piperidine	5.0	7.0	8.6±1.4	+1.0	38.0±17.6	+23.0

I — Inoculated leaves; II — secondarily (systemically) infected leaves.
* TMV titre determined serologically. The values indicate mean dilution steps. Steps 1—10 correspond to dilutions from 1:2 to 1:1024.
** All differences insignificant.

Neither HMPT nor piperidine exerted an effect on f2 phage adsorption on *E. coli* cells (Table 3).

One-step growth experiments revealed a marked difference between HMPT and piperidine. While a 0.1 % HMPT solution delayed the phage release by 20 min on the average, an approximately aequimolar piperidine solution had no effect on the time course of phage replication (Fig. 1).

The results obtained with TMV are summarized in Tables 4 and 5. The serologically assayed TMV contents in inoculated and secondarily infected leaves of *N. tabacum* was not substantially affected either by MHPT or piperidine. Local lesion infectivity assays suggested a certain reduction in TMV concentration in inoculated and HMPT-treated leaves. The virus contents in secondarily infected leaves was not affected by HMPT. Piperidine had no effect on TMV contents in either inoculated or secondarily infected leaves.

Local lesion formation was strongly inhibited by HMPT (to 30 % of the control) and only little by piperidine (Table 5).

In vitro experiments on f2 phage and TMV showed that the action of HMPT was not due to a direct effect on free virions.

Table 5. Effects of HMPT and piperidine on local lesion formation by TMV on N. glutinosa leaves

Treatment	No. of lesions per half leaf absolute	%	Difference to control
None	98.0+10.0	100.0	
HMPT	29.9+ 5.2	30.5	−69.5*
None	27.0+ 5.0	100.0	
Piperidine	19.6+ 5.4	72.6	−27.4**

* and **: Differences significant and insignificant. respectively.

Discussion

In our experiments, HMPT was active against bacteriophages and inhibited local lesion formation by TMV. As to the mechanism of its action, HMPT did not affect f2 phage adsorption, but in one-step growth experiments it delayed phage release by 20 min. This delay itself can hardly explain the drastic reduction in plaque numbers so that an inhibition of virus synthesis should be taken into account. It cannot be excluded that HMPT exerts its effect via the host metabolism, because HMPT concentrations higher than 0.1 % markedly inhibited the replication of host bacteria.

The replication of TMV in a systemic host was not significantly affected by HMPT, but infectivity assays regularly suggested a tendency to a decreased virus contents in the inoculated leaves. This tendency should be stressed in view of the fact that closely related triazole derivatives reduced TMV concentration to one third of the control (Menzel and Kluge, unpublished).

Two explanations of the inhibition yy HMPT of TMV local lesion formation on *N. glutinosa* leaves may be proposed: either a reduction in the number of infectible sites by HMPT or an inhibition of virus replication or spread directly at the site of infection. The present experiments made a decision between these two possibilities impossible.

On the other hand it is probable that the effects of HMPT on RNA phages and TMV were not due to the piperidine moiety of the triazole compound or to piperidine as a possible split product in the host organism. The effect of piperidine, even if used in aequimolar amounts, namely was always markedly lower than that of HMPT.

Acknowledgements. We thank Prof. B. Hirsch, Technical University, Dresden, and Dr. D. Lohmann, VEB Isis-Chemie Zwickau, for the synthesis of HMPT. We also thank Mrs. A. Gentschev for excellent technical assistance.

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