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Short Communication

Enhanced inhibition of plant virus replication by pyridylthiourea compounds complexed with metal ions

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Summary

Inhibition of potato virus X replication can be enhanced by complexation of *N*-allyl-*N'*-2-pyridyl-thiourea and of *N*-phenyl-*N'*-2-pyridyl-thiourea with Cu^+, Zn^{2+} and Cd^{2+}. The antiphytoviral activity of some complexes with pyridylthiourea can be further augmented by adding small quantities of 2,4-dioxohexahydro-1,3,5-triazine.

Antiphytoviral substance; Pyridylthiourea; 2,4-Dioxohexahydro-1,3,5-triazine; Potato virus X

Introduction

Substituted thioureas (TU) inhibit plant, animal and human virus replication (for a survey see Schuster, 1988; Gălăbov et al., 1972). However, augmentation of their antiviral activities seems to be a prerequisite for their practical use. Augmentation of antiphytoviral activity has been demonstrated by combined treatment of virus-infected plants with TU and 2,4-dioxohexahydro-1,3,5-triazine (DHT; Schuster et al., 1979), which is an excellent synergist of a number of TU derivatives (Schuster

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and Vassilev, 1985). The combined use of pyridyl-TU complexes with metals may be another interesting way to enhance the antiphytoviral activity of 2-pyridyl-TU. We also investigated whether the antiphytoviral activity of metal pyridyl-TU complexes could be further enhanced by DHT.

Materials and Methods

Chemicals

Coordination compounds of *N*-allyl-*N'*-2-pyridyl-thiourea (APTU) and *N*-phenyl-*N'*-2-pyridyl-thiourea (PPTU) complexes with Cu^{+}, Zn^{2+} and Cd^{2+} were synthesized at the Higher Institute of Chemical Technology, Bourgas, Bulgaria. A detailed description of the synthesis will be presented elsewhere (Davarsky et al., in preparation).

The IR spectral properties of the coordination compounds demonstrate that in all cases the ligands are bidentate. The complexation with the metals takes place through the sulphur atom of the TU and the nitrogen atom of the pyridine substituent. Fig. 1 demonstrates the proposed structure of the pyridylthiourea derivatives.

2,4-Dioxohexahydro-1,3,5-triazine (DHT) was synthesized in the Chemiekombinat Bitterfeld, G.D.R.-4400 Bitterfeld.

Estimation of the antiphytoviral activity

The virus and host plant employed were the H19 strain of potato virus X (PVX) and *Nicotiana tabacum* L. 'Samsun', respectively. The plants were cultivated in an air-conditioned room at a temperature of 25 ± 2°C during a 16-h light period and

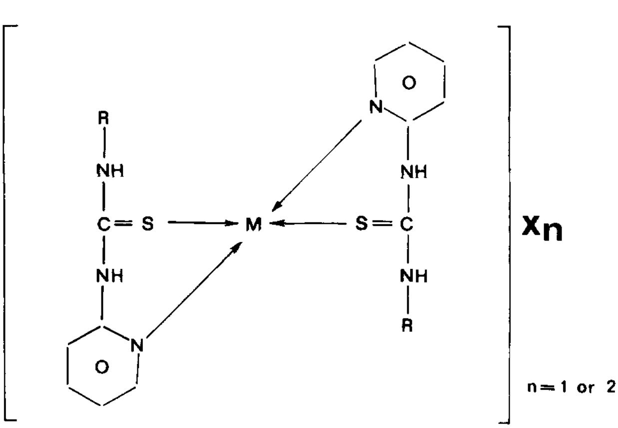

Fig. 1. Proposed structure of the pyridyl thiourea complexes. R = allyl or phenyl; M = Cu^{+}, Zn^{++} or Cd^{++}; X = Cl^{-}, Br^{-}, J^{-} or NO_3^{-}.

TABLE 1

Influence of *N*-allyl-*N*′-2-pyridyl-thiourea (APTU), *N*-phenyl-*N*′-2-pyridyl-thiourea (PPTU) and the corresponding metal complexes on the replication of PVX in inoculated and systemically infected leaves of *Nicotiana tabacum* L. 'Samsun'

Treatment	Concentration (mmol)	Virus content (%)[a,b]	
		inoculated	systemically infected
Control	–	100	100
APTU	5.0	78 (NS)	46^{++}
$Cu(APTU)_2Cl$	0.5	52^{+}	57^{+}
$Cu(APTU)_2Br$	0.5	54^{+}	15^{+++}
$Zn(APTU)_2Cl_2$	0.5	74 (NS)	26^{+++}
$Cd(APTU)_2Cl_2$	0.5	40^{++}	3^{+++}
PPTU	5.0	81^{+}	74^{+}
$Cu(PPTU)_2Cl$	5.0	50^{+}	69^{+}
$Zn(PPTU)_2Cl_2$	5.0	70^{+}	55^{+}
$Zn(PPTU)_2Br_2$	5.0	85 (NS)	41^{+}
$Zn(PPTU)_2J_2$	5.0	87 (NS)	33^{++}
$Cd(PPTU)_2Cl_2$	5.0	108 (NS)	44^{+++}
$Cd(PPTU)_2J_2$	5.0	82 (NS)	31^{+++}

[a]Virus content is indicated in percent of that found in the infected untreated controls.
[b]Statistical significance: $^{+}$, $P \leqslant 0.05$; $^{++}$, $P \leqslant 0.01$; $^{+++}$, $P \leqslant 0.001$ or NS, not significant.

at 20 ± 2°C during an 8-h dark period. When the plants had developed 5 to 6 leaves, two of the lower leaves were inoculated with infective sap obtained from PVX-infected *N. tabacum* 'Samsun' plants. The compounds being tested were applied

TABLE 2

Stimulation of the antiphytoviral activity of *N*-allyl-*N*′-2-pyridyl-TU (APTU), *N*-phenyl-*N*′-2-pyridyl-TU (PPTU) and the corresponding metal complexes by 2,4-dioxohexahydro-1,3,5-triazine (DHT) in systemically infected leaves of *Nicotiana tabacum* L. 'Samsun'

Treatment	Concentration (mmol)	DHT Concentration (mmol)	Virus content (%)[a,b]		
			TU alone	DHT alone	Combination of TU and DHT
Control	–	–	100	100	100
APTU	5.0	0.4	46^{++}	26^{+++}	8^{+++}
$Cu(APTU)_2Br$	0.5	0.5	27^{++}	7^{+++}	0^{+++}
$Zn(APTU)_2Cl_2$	0.5	0.5	26^{+++}	4^{+++}	0^{+++}
$Cd(APTU)_2Br_2$	0.5	0.4	88 (NS)	47^{++}	16^{+++}
$Cd(APTU)_2(NO_3)_2$	5.0	0.4	122 (NS)	55^{++}	9^{+++}
PPTU	5.0	0.5	74^{+}	4^{+++}	3^{+++}
$Cu(PPTU)_2Cl$	1.0	0.5	94 (NS)	7^{+++}	2^{+++}
$Cu(PPTU)_2Br$	1.0	0.5	100 (NS)	7^{+++}	0^{+++}

[a]Virus content is indicated in percent of that found in the infected untreated controls.
[b]Statistical significance: $^{+}$, $P \leqslant 0.05$; $^{++}$, $P \leqslant 0.01$; $^{+++}$, $P \leqslant 0.001$ or NS, not significant.

in aqueous solutions by spraying (until run-off) at 2 days before and 2 and 7 days after inoculation. The optimum concentrations of the complexed pyridylthiourea compounds, which did not damage the plants, had been established in preceding experiments and used in those reported here (Tables 1 and 2). Tween 80 (0.125%) was used as solvent and Fekama adhesive (0.2%) as spreader sticker. The virus-inoculated control plants were treated with water containing the same concentrations of Tween 80 and Fekama as the solutions with pyridylthiourea complexes.

The virus concentration was assayed by dilution endpoint determination in the precipitation drop test. The uppermost inoculated leaf and the systemically infected third leaf above it were tested on the 5th and 13th day after inoculation, respectively. Each of the 8 to 10 plants, used in one test, was assayed separately. The two-fold dilution step at which no precipitate was observed was used as an index (expressed as an exponent of 2). Average indices were calculated for each series. Shown in Tables 1 and 2 are the average virus concentrations (indices) of the treated plants relative to the average virus concentrations of the corresponding untreated controls. The lower these values, the higher are the antiphytoviral activities of the corresponding treatments. The significance of the differences was assessed by a single tail *t*-test (Mudra, 1958). If necessary, the results of the serological assay were remodelled by suitable transformations in order to obtain normal distributions and classes of unitary magnitude (Grimm, 1960).

The experimental procedures and calculations are described in detail by Schuster (1976) and Schuster and Vassilev (1983).

Results and Discussion

Treatment with metal complexed compounds inhibited the replication of PVX in inoculated and systemically infected leaves of *N. tabacum* 'Samsun' more effectively than treatment with non-complexed APTU or PPTU (Table 1). The most active APTU complexes affected virus replication at a concentration that was one tenth of the molar concentration of APTU. For instance, PVX content in systemically infected leaves was reduced to 3% following treatment with 0.5 mmol $Cd(APTU)_2Cl_2$. After treatment with 0.5 mmol $Cu(APTU)_2Br$ virus content was reduced to 15%, whereas treatment with 5 mmol APTU resulted in a virus content that was 46% of that of the control. In inoculated leaves the corresponding values were 40, 54 and 78%, respectively. This demonstrates the strong antiphytoviral effect of appropriate coordination complexes of pyridylthiourea. That the inhibition effects were more pronounced in systemically infected than in inoculated leaves, is in agreement with previous observations on ribavirin (Schuster, 1976), 1,3,4-thiadiazoles (Schuster et al., 1984), 1,3-oxazoles (Schuster and Ulbricht, 1985) and other substances. Ribavirin gave similar results in systemically infected and inoculated leaves, if the tissue disks were infiltrated immediately after inoculation. The rate of adsorption of ribavirin through the epidermis and the lower replication rate in tissue disks, as compared with whole leaves, may play a determinant role (Schuster and Huber, unpublished data). Also, the influence of antiviral substances on the speed of systemic virus spread must be taken into consideration. This is particularly true for *m*-guanidino-acetophenone-(4,5-diphenyl-oxazole-2-yl-hydrazone)-hydrochloride and some other oxazoles (Schuster and Ulbricht, 1985).

Usually, combined treatment of lower doses of metal thiourea complexes and DHT resulted in a greater inhibition of PVX replication (Table 2, column 6) than treatment with TU (column 4) or DHT (column 5) alone. Some combinations of metal thiourea complexes with DHT inhibited virus replication to such extent that PVX was no longer detectable. $Cd(APTU)_2Br_2$, $Cd(APTU)_2(NO_3)_2$ or $Cu(PPTU)_2Br$ combined with DHT resulted in a strong inhibition, whereas the coordination compounds applied alone had no or only low inhibitory effect.

Thus, the antiphytoviral activities of the metal complexes can be further augmented by small doses of DHT. The mechanism of the enhancing effect of the metals on the antiphytoviral activity of thiourea and of the further enhancement of this activity upon addition of DHT is unknown. The interesting possibility that the activity of thiourea compounds against animal or human viruses can be potentiated in a similar manner must also be explored. The increased antiviral activity of thiourea metal complexes warrants further investigations in plant, animal, human and other virus systems.

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