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ANTIVIRAL ACTIVITY OF α-(METHYL-PHTHALIMIDO)-α′-(SUBSTITUTED STYRYL)-CYCLOHEXANONE-THIOSEMICARBAZONES

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ONE of the areas where most encouraging antiviral activity has been encountered is that of thiosemicarbazones. The first report by Hamre and coworkers[1] demonstrated that *p*-amino-benzaldehyde thiosemicarbazones showed a significant antiviral activity. As a result of enhanced antiviral activity of thiosemicarbazone derivatives, a number of thiosemicarbazones incorporating various heterocyclic and polynuclear were screened for trial and a few exhibited remarkable antiviral activity[2-8]. The most effective and potent compound of this series is Methisazone or Marboran. This compound, chemically, N-methyl-isatin-β-thiosemicarbazone has been found to exhibit pronounced antiviral activity[9,10].

In view of the structural analogy, a few new thiosemicarbazone derivatives have been synthesised in the hope that the presence of thiosemicarbazone moiety in this class of compounds might render improved therapeutic results.

Antiviral screening against Sunnhemp rosette virus (SRV): The antiviral screening of the synthesised compounds was evaluated against the SRV both *in vitro* and *in vivo* by the method of Verma *et al*[11] in the plant *Cyamopsis tetragonoloba*.

Maintenance of virus culture and preparation of the virus inoculum: The culture of SRV was maintained on the systemic host *Crotolaria juncea*.

The virus inoculum was prepared by grinding young diseased leaves of *C. juncea* in a sterilized pestle and mortar using distilled water as a diluent (1 ml/g). The pulp so obtained was squeezed through two folds of muslin cloth and the filtrate centrifuged at 3000 g for 15 min. The resultant partially clarified supernatant thus obtained was diluted suitably with distilled water and used as a virus inoculum.

The solutions of the test compounds were prepared by dissolving 2 mg of the compound in 1 ml of ethanol and making up the volume to 4 ml with distilled water.

In-vitro activity: In *in-vitro* experiments, 1 ml of the test solution was mixed with 1 ml of the virus inoculum and incubated for 30 min, and then spread over the leaves of *C. tetragonoloba*. An equal number of leaves were rubbed with a mixture of virus and distilled water and served as controls.

In-vivo activity: To detect the viral inhibitory activity the compound was applied on the two basal leaves of the test plant *i.e. C. tetragonoloba*, whereas control sets were treated with a mixture of ethanol (1 ml) and distilled water (4 ml).

After an interval of 24 hr. the leaves of the test plant were washed with distilled water, blotted dry, dusted with 600 mesh carborundum powder and inoculated with the virus inoculum SRV. All the fully expanded leaves of hypersensitive hosts were inoculated with the virus.

At least five plants, with four leaves being of equal size were used in each experiment.

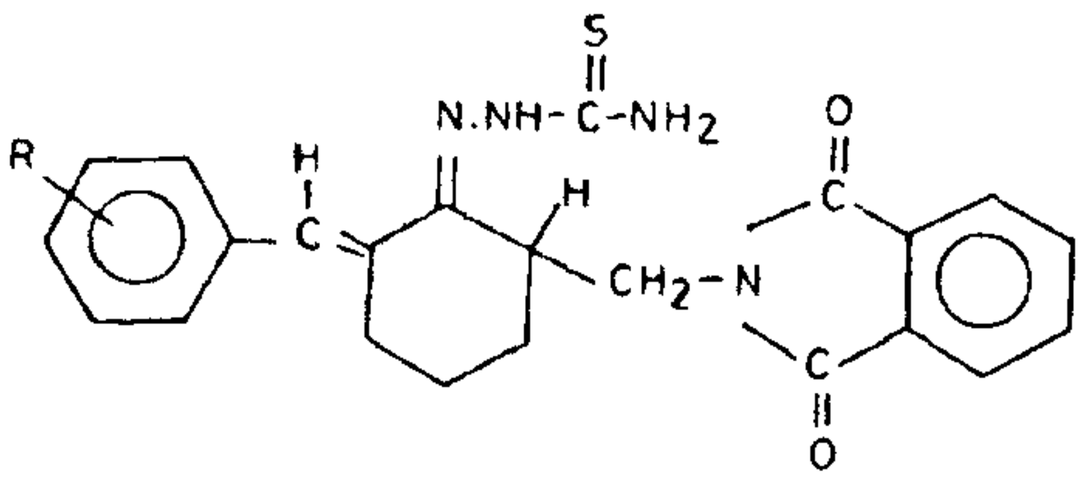
All the experiments were performed in insect-free glass house, kept at about 20–30°C. The phytotoxic symptoms, if any, and the number of local lesions were observed throughout the experiment. Local lesions were counted 3–4 days after virus inoculation. The percentage inhibition was calculated by the formula % inhibition $= [(C-T)/C] \times 100$ where C is the number of lesions in control plant and T is the number of lesions in treated plant. The activity exhibited by compounds against Sunnhemp rosette virus has been given in table 1.

The compounds exhibited broad spectrum antiviral activity against SRV both *in vitro* as well as *in vivo* in the same host *C. tetragonoloba*.

It is apparent from the result recorded in table 1 that when R = 2-OH, 1-CH=CH- and 4-Cl then the percentage inhibition against SRV is quite high. Also, N-(methyl phthalimido) cyclohexanone thiosemicarbazone shows 70% inhibition, indicating that the derivatives of this compound are less active than the parent moiety.

These thiosemicarbazones are more active *in vivo* against SRV than *in vitro*. When R = 2-OH and 1-CH=CH-, the compounds show 62 and 68% in-

Table 1 *Antiviral activity of α-(methyl phthalimido)-α-(substituted styryl)-cyclohexanone-thiosemicarbazones against the Sunnhemp rosette virus*



Compound No	R	Percent inhibition of SRV In-vitro	In-vivo
1.	H	0	18[b]
2.	2-OH	59[a]	62[a]
3	4-OCH_3	38[b]	32[b]
4	2-OH,5-OCH_3	23[b]	40[b]
5	2-F	20[b]	43[b]
6	4-$(CH_3)_2N$	20[b]	47[b]
7.	4-Cl	52[a]	40[b]
8.	1-CH=CH-	64[a]	68[a]
9.	Styryl = CH_2	70[a]	42[b]

The concentration of compound used was 2 mg/ml.
Virus: Sunnhemp rosette virus
Test plant: *Cyamopsis tetragonoloba*
Data significance at 1 % level = ([a])
Data significance at 5 % level = ([b])

hibition respectively. But inhibition in the case of 4-Cl is decreased.

The *in vivo* activity of N-(methyl phthalimido)-cyclohexanone thiosemicarbazone is also decreased upto 42 percent. But in general percentage inhibition increases remarkably.

It is interesting to observe that the substituent (R, -CH=CH) profoundly increases the antiviral activity *in vivo* and *in vitro* both.

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A NEW ANTHRAQUINONE PIGMENT FROM THE STEM BARK OF *DIOSPYROS DISCOLOR*

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EVIDENCE is presented of the isolation and characterization of a new anthraquinone glycoside from the stem bark of *Diospyros discolor*. The structure was assigned as 1,3,5,6-tetra-hydroxy-2-methylanthraquinone-8-0-β-D-glucopyranoside by spectroscopic and chemical methods.

The compound gave green colour with $FeCl_3$, positive Borntrager reaction[1] and positive Molisch's test for an anthraquinone glycoside. The UV spectrum (EtOH) of the compound showed absorptions at 230, 280 and 430 nm and its IR spectrum (KBr) exhibited absorptions at 3350–3400 (br, OH), 2910, 1635, 1610, 1580, 1440, 1290, 1120, 1090, 825, 820 and 750 cm^{-1}. Acid hydrolysis of the compound (7 % H_2SO_4) yielded an aglycone and D-glucose. The sugar was identified by direct comparison (co-pc) with an authentic sample and also by preparation of osazone derivative (mp,