NOTES

CORRELATION BETWEEN THE ANTI-ANIMAL AND ANTI-PLANT-VIRUS ACTIVITIES OF SEVERAL ANTIBIOTICS

(STUDIES ON ANTIVIRAL AND ANTITUMOR ANTIBIOTICS. XIX)

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In preceeding papers, we reported on the anti-animal-virus activities of trichothecin[1], verrucarin A[2], brefeldin A[2], ascochlorin[3], xanthocillin X mono- and dimethylether[4~6], methoxy-xanthocillin X dimethylether[4,5], quinomycin B[7], and geodin[8]. Of these antibiotics, only trichothecin is known to inhibit plant virus multiplication[9]. Since there was a possibility that some of the anti-animal-virus antibiotics might affect plant virus growth, the effect of the above cited antibiotics on tobacco mosaic virus (TMV) was examined employing the *in vitro* local lesion method. Our results show that a rather intimate relationship existed between the two activities and the correlation is discussed in this paper.

Trichothecin and verrucarin A have the trichothecolone moiety in common. Trichothecolone was also found to be active against Newcastle disease virus (NDV) as determined by the agar-diffusion plaque-inhibition method, but its minimum inhibitory concentration was higher than that of trichothecin or verrucarin A (Table 1). These three compounds effectively suppressed local lesion formation on Pinto bean leaf discs infected with TMV, and complete inhibition was observed with trichothecin at non-phytotoxic concenctrations (Table 2). The minimum inhibitory concentration of trichothecolone was found to be 250-fold higher than that of trichothecin or verrucarin A alone. This ratio was much higher than that found in anti-animal-virus activity. Verrucarin A was also very toxic for cultured chick embryo cells, and the minimum inhibitory concentration determined by the agar-diffusion plaque-inhibition method was far lower in comparison with trichothecin (Table 1). In contrast to this observation, the effects on TMV and host plants of the two antibiotics were similar, *i. e.*, the concentration required for 50 % reduction of the number of necrotic spots and the maximum concentration tolerated by the plant were nearly the same with the two antibiotics.

Brefeldin A has lower effective concentration against NDV than trichothecin, and its toxicity for animal cells as determined by the agar-diffusion method is rather weak[2]. The phytotoxicity of the two antibiotics was in good agreement with the cytotoxicity (Tables 2 and 3). But brefeldin A inhibited local lesion formation on Pinto bean leaf discs at a much higher concentration than trichothecin.

Quinomycin B and geodin are very cytotoxic antibiotics, and they suppressed plaque-formation by NDV at sub-cytotoxic concentrations[7,8]. The same was observed in the case of anti-plant-virus activity (Table 3). The decrease in the number of necrotic spots on TMV-infected leaf discs was proportional to the antibiotic concentrations, but complete inhibition was not observed at sub-phytotoxic concentrations.

Ascochlorin, xanthocillin X mono- and dimethylethers, and methoxy-xanthocillin X dimethylether were not active on the multiplication of TMV, and also did not show phytotoxicity at the highest concentration examined (Table 3). Both trichothecin and xanthocillin X monomethylether are inhibitors of protein synthesis[6,10]. Ascochlorin

Table 1. Anti-NDV activity of trichothecin, verrucarin A and trichothecolone as determined by the agar-diffusion plaque-inhibition method.

Concentration (μg/ml)	Trichothecin		Verrucarin A		Trichothecolone	
	CTZ (mm)	AVZ (mm)	CTZ (mm)	AVZ (mm)	CTZ (mm)	AVZ (mm)
4,000	N.D.*	N.D.	N.D.	N.D.	—	38.4
2,000	N.D.	N.D.	N.D.	N.D.	—	36.5
1,000	41.8	60.7	50.2	63.7	—	24.7
500	31.4	45.2	42.7	55.0	—	15.7
250	25.9	43.6	35.0	47.8	—	13.8
125	±	35.2	33.6	45.4	—	10.2
62.5	—	28.8	30.5	43.0	—	—
31.3	—	24.0	28.6	38.8	—	—
15.6	—	16.2	23.2	32.1	—	—
7.8	—	13.2	23.0	30.5	—	—
3.9	—	10.8	22.5	29.3	—	—
2.0	—	—	17.2	22.6	—	—
1.0	—	—	14.6	21.4	—	—
0.5	—	—	10.5	17.0	—	—
0.25	—	—	—	14.5	—	—
0.12	—	—	—	12.2	—	—
0.06	—	—	—	10.9	—	—

* N.D. : Not determined.

Confluent monolayer cultures of chick embryo fibroblasts in Petri dishes were infected with NDV as described previously[4]. Paper discs (8 mm in diameter, absorbed *ca.* 0.025 ml drug solution/disc) impregnated with the drug solutions were placed on hardened soft-agar overlayers. After 2-day incubation at 40℃, diameters of cytotoxic zone (CTZ) caused by the drug and antiviral zone (AVZ) where plaque-formation was suppressed were measured.

Table 2. Anti-TMV activity and phytotoxicity of trichothecin, verrucarin A and trichothecolone as determined by the local lesion method (*in vitro*)

Compounds	Concentration (μg/ml)	Inhibition (%)	Phytotoxicity*
Trichothecin	100	100	+
	20	100	+
	4.0	100	±
	0.8	100	—
	0.16	100	—
	0.032	78	—
	0.0064	10	—
	0.0013	0	—
Verrucarin A	20	100	+
	4.0	100	±
	0.8	96	±
	0.16	88	—
	0.032	67	—
	0.0064	25	—
	0.0013	0	—
Trichothecolone	100	100	+
	20	74	±
	4.0	58	—
	0.8	24	—
	0.16	0	—
	0.032	0	—

* — : no phytotoxicity, ± : slight phytotoxicity, and + : severe phytotoxicity.

Twenty to 25-day-grown bean (*Phaseolus vulgaris* L., 'Pinto') was used as a test plant. Leaf discs, 12 mm in diameter, were prepared by punching out bean coleoptiles preinoculated with TMV using carborundum and a cotton swab. Inoculum was a crude solution extracted from TMV-infected tobacco leaves (*Nicotiana tobacum* L., Bright yellow). These discs were immersed in various concentrations of antibiotics in small Petri dishes (3 cm in diameter) and incubated at 21℃ under continuous fluorescent illumination. After 3-day incubation, the number of necrotic spots developed on the leaf discs of bean was counted and the inhibition rates were calculated and expressed as percent.

has been shown to be a potent inhibitor of mitochondrial respiration, and some other respiration inhibitors such as rotenone and the related compounds were found to be active on both animal and plant viruses[11,12]. These differences in the activities on animal and plant viruses may be caused by the difference in their action mechanisms and more possibly, by the structural differences in the animal and plant cells as discussed below.

Blasticidin S, bihoromycin, cycloheximide, puromycin, and formycin B (laurusin) have been reported to be active on plant viruses (see review by SHIMOMURA & HIRAI. ref. 13). Blasticidin S, cycloheximide, puromycin and formycin B are known to inhibit the growth of NDV[6]. Bihoromycin was found to suppress plaque formation by NDV as shown in Table 4. Thus, all the well known anti-plant-virus antibiotics are also active in some way on an animal virus, NDV. NDV and plant viruses are RNA viruses. But the reverse is not always true as indicated in the cases of ascochlorin, xanthocillin X mono- and dimethylethers, and methoxy-xanthocillin X dimethylether (Table 3). Plant leaves have a cuticle layer at their surface but cultured animal cells do not have such a protective layer. This difference may profoundly affect the permeability of antibiotics, and may partly explain the differences in activities of antibiotics when tested against animal and plant viruses.

Despite the differences in host cells, a

Table 3. Effect of brefeldin A, quinomycin B, geodin, ascochlorin, xanthocillin X mono- and dimethylether, and methoxy-xanthocillin X dimethylether on local lesion formation of TMV-infected bean (*in vitro*).

Antibiotics	Concentration (μg/ml)	Inhibition (%)	Phytotoxicity*
Brefeldin A	100	100	±
	20	89	±
	4.0	32	−
	0.8	10	−
	0.16	0	−
Geodin	100	95	+
	20	58	−
	4.0	31	−
	0.8	0	−
Quinomycin B	100	98	+
	20	74	±
	4.0	60	±
	0.8	0	±
Ascochlorin	100	0	−
Xanthocillin X monomethylether	100	0	−
Xanthocillin X dimethylether	100	0	−
Methoxy-xanthocillin X dimethylether	100	0	−

* −: no phytotoxicity, ±: slight phytotoxicity, and +: severe phytotoxicity.

Table 4. Anti-NDV activity of bihoromycin as determined by the agar-diffusion plaque-inhibition method.

Bihoromycin concentration (μg/ml)	CTZ* (mm)	AVZ* (mm)
36	+**	43.6
18	+	38.7
9.0	+	38.5
4.5	+	36.0
2.2	+	35.0
1.1	+	31.5
0.56	+	24.3
0.28	−	23.5
0.14	−	21.0
0.07	−	17.7

* Same as in Table 1.
** Boundaries between CTZ and AVZ were not clear and measurement was impossible.

rather intimate correlation was observed between anti-animal and anti-plant-virus activities, *i.e.*, 40 out of 47 compounds including the above mentioned antibiotics which inhibit NDV growth *in vitro* also partially or completely suppressed necrosis following TMV infection at concentrations below 100 mcg/ml.

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